TAIBAH UNIVERSITY

COLLEGE OF PHARMACY

Department of Pharmaceutics&

Pharmaceutical Technology

Handbook on Practical

Pharmaceutical Microbiology I

Contents

1- Safety management.
2- Identification of bacteria under microscopy and Smear Preparation.
3- Simple stain.
4- Gram-positive bacteria stain (Staphylococcus, Sarcina and Bacillus).

5- Gram-negative bacteria stain (Pseudomonas, E. coli, Neisseria).
6- Mixture of Gram-positive and Gram-negative bacteria stain .
7- Identification of candida albicans.
8- Ziehl-Neelsen Stain For Acid-Fast microorganism (T. B.)
9- Staining of spores (Bacillus).
10- Wide distribution of microorganisms, Distribution of microorganisms in environment.
11- Different culture media.
12- Streaking techniques.
13- Isolation of pure colonies on nutrient agar and on blood agar.
14- Determination of Viable Count of microorganisms.
15- Assay of an antibiotic against standard antibiotics.

Module 1

RULES AND SAFETY MEASURES IN MICROBIOLOGICAL LABORATORY.

Rules and Safety Measures in Microbiological Laboratory

- It is very important to follows rules of microbiological laboratory in safety point of view.
- If these important rules are not followed a student,teacher, or a laboratory worker can face Biohazards, Chemical hazards as well as Physical hazards.

The safety rules are as follows

- Whenever a person enters a laboratory, he/she should remove the footwear out of the laboratory.
- Dyes and chemicals are used in the laboratory due to which our skin can be harmed as well as it can damage our clothes so laboratory gown or apron should be used compulsory.
- Instruction given by the instruction should be noted and followed carefully.
- Eating and drinking are not allowed in laboratory.
- Do not put anything in your mouth like pen, pencils, fingers etc.
- Hair should be packed with hair cap to avoid falling of hair on face, working place as well as burning Bunsen burner.
- Laboratory doors and windows should be kept closed during experiments are going on.
- Extra material like bags, clothes, purses and books should be kept at proper places like drawers or shelf’s.
- The working area should be kept free from all these extra material.
- Before starting your work on your work bench the bench top should be disinfected by ethanol, phenol, Lysol or cetylpyridinium chloride before and after each laboratory work.
- Hands should be washed properly before and after each task.

- These above precaution may lessen chances of infection or contamination due to cultures.
- Smoking is prohibited completely in microbial laboratory.
- Microbial cultures should be handled properly and should not be taken out of laboratory.
- Mouth pipetting should be avoided and mechanical pipetting devices should be used.
- All laboratory work should be carried out carefully to avoid aerosol formation.
- Talking, laughing should be avoided because it can result into contamination.
- All contaminated material should be placed into a proper container.
- Culture plates, tubes should be discarded in basket.
- All microbial cultures should be either incubated or refrigerated. Cultures should not be kept in drawers, cupboard or desk.
- Pipettes, glass rods should be properly disinfected or sterilized after each use.
- Before and after each use; wire loop should be sterilized and then used or kept.
- All laboratory instruments should be operated as per standard operating procedure.
- Bunsen burner are very dangerous so they should be handled with care and should be turned off when not in use.
- All electrical appliances such as oven, autoclave, centrifuge and other such devices should be immediately turned off after each use.
- All accidents, injuries or broken glassware should be reported immediately.
- Alcohol should be kept away from flame.
- Waste paper, threads, broken glassware or used pH strips should be discarded safely.
- The experiments that are permitted should only carried out.
- Keep the laboratory clean.
- Follow all the instruction above and you will be safe and get good results.

Exercise 1

State the following statements are TRUE or FALSE:

1. Eating and drinking are allowed in laboratory.
2. Alcohol should be kept away from flame.
3. Pipettes, glass rods should be properly disinfected or sterilized after each use.
4. Before each use only; wire loop should be sterilized and then used or kept.
5. All microbial cultures should be kept in drawers, cupboard or desk.
6. Culture plates, tubes should be discarded in basket.
7. Smoking is allowed in microbial laboratory.
8. Mouth pipetting are allowed.
9. Extra material like bags, clothes, purses and books should be kept at proper places like drawers or shelf's.
10. Hands should be washed properly before and after each task.

Module 2

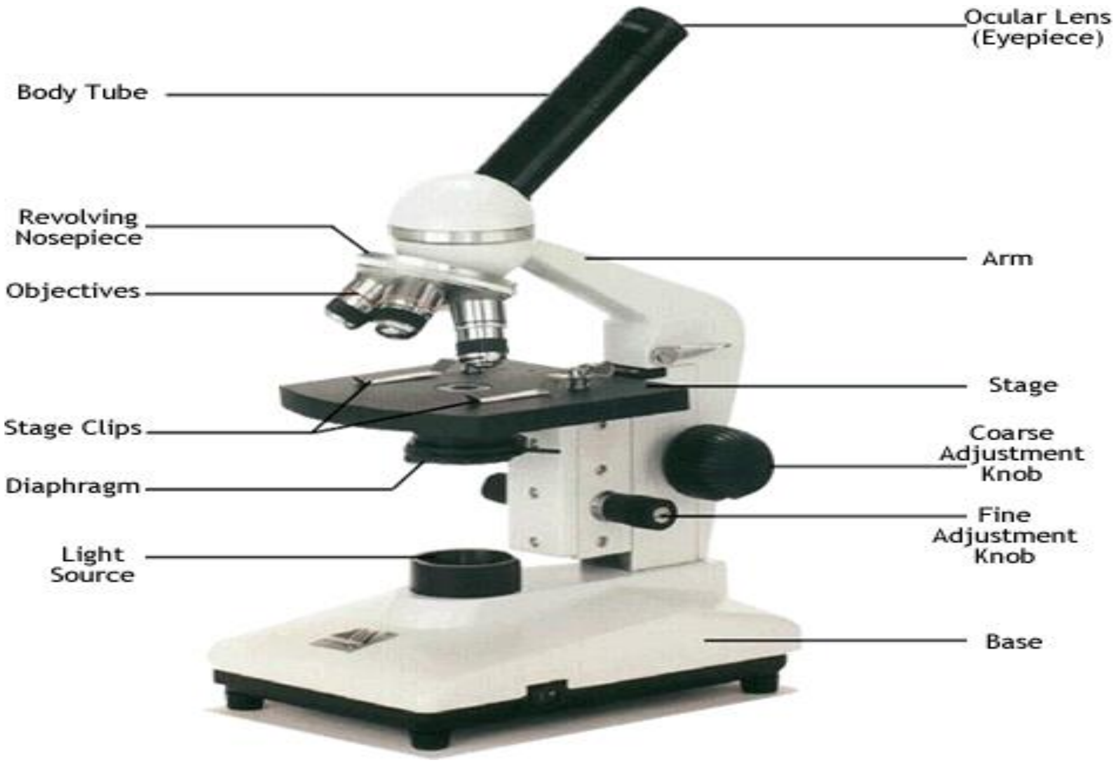
IDENTIFICATION OF BACTERIA UNDER MICROSCOPY

Microbiolog means:

“Micro” means: Tiny , small, where “Biology” means science.

Therefore, the definition of Microbiology is “It is the science dealing with study of organisms that cannot be seen by naked eye.”

Parts of compound microscope



Parts of the light microscope

1. Arm and base. All other parts of the microscope are attached to the arm or base.

2. Mechanical stage: The platform on which the microscope slide rests and the clamping device that secures the slide.

3. Mechanical stage control knobs (x-y control knobs): These knobs, under the stage, move the stage front to back and the slide from side to side.

4. Lamp (illuminator). The lamp on/off switch is located on the base. The light intensity can be adjusted with this on/off switch. The lamp should be adjusted to a medium level at the start of viewing. The lamp or illuminator usually has a blue filter that rests on the light housing or under the condenser.

5. Iris diaphragm: adjusts the amount of light reaching the specimen. It is adjusted with a thin, black lever under the stage. It has a dramatic effect on the contrast observed in the specimen and may need to be adjusted frequently.

6. Lens systems: There are three lens systems: the eyepieces (ocular), the objectives (four), and the condenser.

    1) Eyepieces (ocular): Magnification of 10X. The eyepieces are held rather loosely in the eyepiece tubes. Never remove the eyepieces from the eyepiece tubes. A rubber eye shield should be on the top of each eyepiece. The width between the eyepieces should be moved until a full circle (the viewing field) is visible with both eyes simultaneously.

    2) Objectives: There are four objectives: 4X (red), 10X (yellow), 40X (blue), and 100X (white, oil immersion objective). The objectives are attached to a rotating nosepiece (nose turret).

The total magnification is calculated by multiplying the ocular magnification and the magnification of the objective in use.

3) Condenser: The condenser is located directly beneath the stage. It gathers and conducts the light to the specimen. Although it can be raised and lowered with the condenser adjustment knob, the condenser should remain at its highest position.

7. Focusing knobs: located on both sides of the microscope.

The larger, inner coarse adjustment knob moves the stage up and down much faster and farther than the smaller, outer fine adjustment knob. The coarse adjustment knob is used ONLY with the low power (4X, 10X) objectives. When focusing under the 40X or 100X objective, ONLY use the fine adjustment, never the coarse adjustment.

8. Nosepiece: Holds the objective lenses

Principle of the microscope

When light passes from a material of one refractive index to another (for example: from glass to air), it bends. In the space between the microscope objective lens and the slide (where air is), light is refracted, the light scatters and it is lost. The refractive index of air is approximately 1.0, while the refractive index of glass is approximately 1.5. When light passes through both glass and air it is refracted. Light of different wavelengths bends at different angles, so as objects are magnified more, images become less distinct.

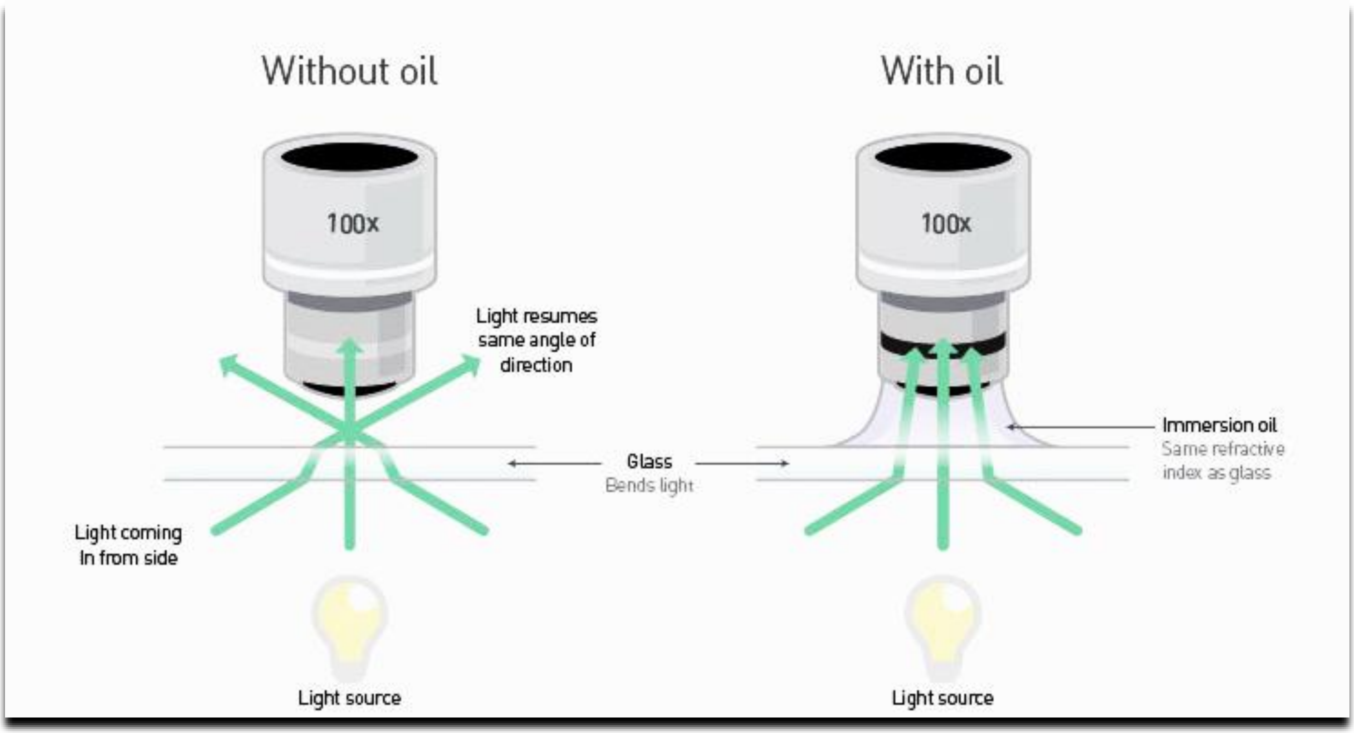
Basically when using lower magnification microscope objective lenses (4x, 10x, 40x) the light refraction is not usually noticeable. However, once you use the 100x objective lens, the light refraction when using a dry lens is noticeable. If you can reduce the amount of light refraction, more light passing through the microscope slide will be directed through the very narrow diameter of a higher power objective lens. In microscopy, more light = clear and crisp images. By placing a substance such as immersion oil with a refractive index equal to that of the glass slide in the space filled with air, more light is directed through the objective and a clearer image is observed.

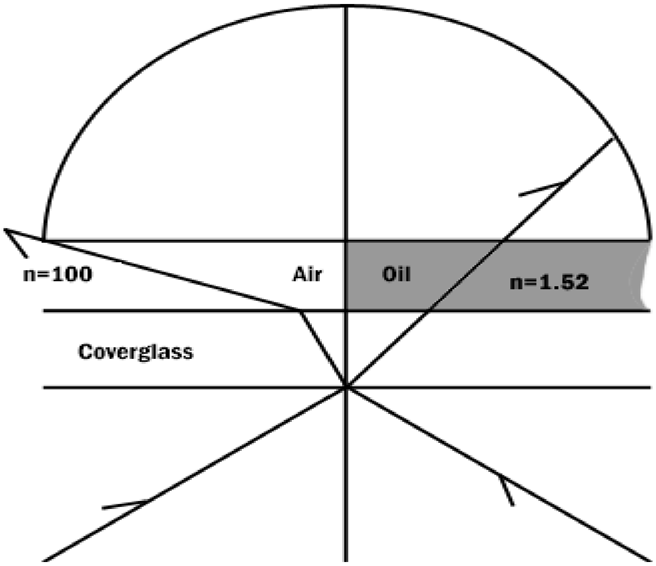
Therefore, oil immersion is a technique used to increase the resolving power of a microscope. This is achieved by immersing both the objective lens and the specimen in a transparent oil of high refractive index, thereby increasing the numerical aperture of the objective lens.

The objective lens used in microbiology is the oil immersion lens (100 x).

Magnification power of microscope = M. P. of ocular lens x M. P. of objective lens.

In order to overcome the difference of refractive index between glass (n=1.52) and air (n=1); cedar wood oil (n=1.52) is used.


Without oil
With oil
100x
100x
Light resumes same angle of direction
Immersion oil
Same refractive index as glass
Glass
Bends light
Light coming In from side
Light source
Light source



Natural Immersion Oils

Cedar Wood Oil

Cedar Wood oil was the immersion oil of choice for many years before the large scale manufacture of synthetic alternatives. And you can still buy Cedar Wood oil today. However, this oil can have many disadvantages. If not correctly cleaned up after use, it can penetrate and damage the cement which holds the objective front lens in place. Cedar wood oil can also ‘yellow’ with age and has a tendency to absorb light in the ultraviolet and blue range of the spectrum.

How to use microscope immersion oil?

1. Place your sample on the microscope stage, and center it using the 4x objective.

2. Once centered, gradually work your way up through the objectives.

Gradually moving up in magnification allows you to fine tune the positioning of the sample, ensuring that the desired element of your sample will be centered in the image when you make it to the 100x objective.

3. When you reach the 100x objective, raise the objective up, and place a drop of immersion oil on top of the cover slip.

The bead of oil should be small enough to fit on the slide, but large enough to cover your sample, and immerse the lens tip.

The drop should be thick enough so that it beads up, so not so thin that the lens cannot be immersed in it. The lens, in this situation, is referring to just the glass tip of the 100x objective.

4. Slowly adjust the focusing knobs to bring the lens to be just immersed in the oil drop.

You don’t want to break the slide, so be careful with your adjustments. The focal point is very, very close for a 100x objective, AND you have to have it immersed in the oil. So there’s a lot of things to pay attention to at the same time.

It may take you a few tries if it’s your first time. If you need to try again (because the oil smeared, for example), simply wipe off the oil from both the objective lens and the cover slip (carefully to avoid damaging your mounted sample). Don’t let the oil dry on either of them, but especially the objective lens–that will take a little more finesse to clean off, but all is not lost unless it gets *inside* your objective somehow.

5. View your sample, using only the fine focus to make minor adjustments.

If you adjust it too much when it’s oil immersed, you could break out of the oil, or smear it. Worse case, you may break your slide and have to start over from the beginning of the whole process.

How to Prepare a Microscope Slide of Bacteria?

- Most of bacteria have no color so they generate little contrast in the microscope field. Therefore, to see bacteria with microscope, it is necessary to apply color by using staining reagent. Once stained; the bacteria may be observed and studied with respect to their shape, size and arrangement.

- A bacterial smear: is a thin layer of bacteria placed on a slide for staining.

- The purpose of making a smear is to fix the bacteria onto the slide and to prevent the sample from being lost during a staining procedure. A smear can be prepared from a solid or broth medium.

Procedure:

1- With wax marking pencil, mark off areas of the slide where the smear will be placed. Place one needle of solid bacterial growth or two loops of liquid bacterial growth in the center of a clean slide.

2. If working from a solid medium, add one drop (and only one drop) of water to your specimen with a water bottle. If using a broth medium, do not add the water.

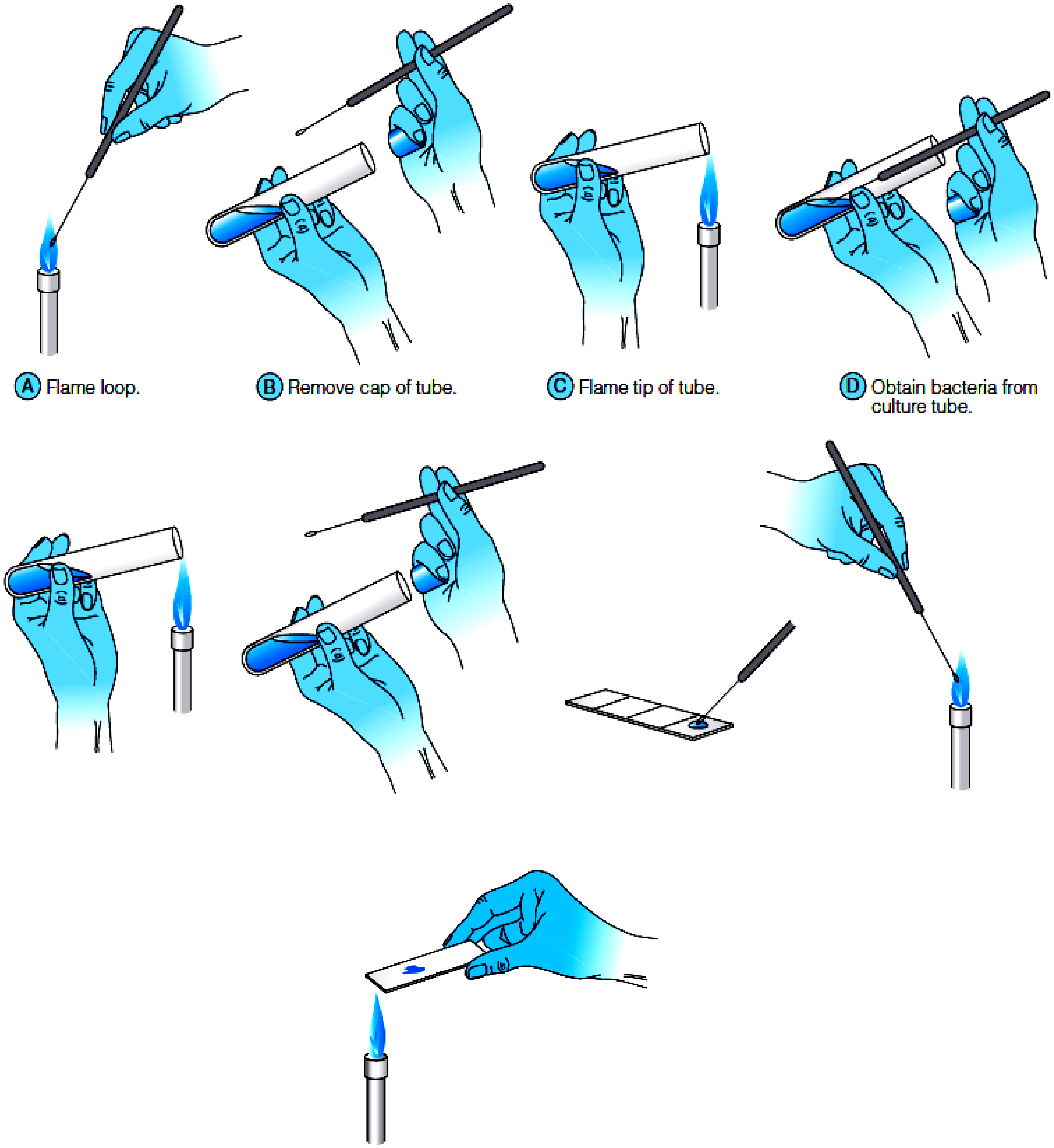
3. Now, with your inoculating loop, mix the specimen with the water completely and spread the mixture out to cover about half of the total slide area.

4. Place the slide on a slide warmer and wait for it to dry. Then heat fix; the smears by quickly passing the slide through the Bunsen burner flame three times. The heat should contact the underside of the slide.

This procedure will

1. Kill any bacteria above that may still alive.

2. Facilitate stain penetration and3) fix cells to the slide so they don't wash off when stained. The smear is now ready for the staining procedure.


(A) Flame loop.
(B) Remove cap of tube.
(C) Flame tip of tube.
(D) Obtain bacteria from culture tube.

Exercise 2

1. Calculate resolution of 10X objective lens ______________

2. Calculate resolution of 40X objective lens ______________

3. What is the purpose of using oil immersion lens?

4. Give examples of oils used with oil immersion lens

5. What is the purpose of heat fixation in preparation of microscopic slide of bacteria?

Module 3

SIMPLE STAIN

Types of stains

a- Simple stain

- Using single dye, which may be

1) Basic dyes:

- e.g.: Crystal Violet, saffranin and methylene blue.
- Bind with COOH gps of amino acids in the cell wall.

2) Acidic dyes:

- e.g.: eosin, acid fuchsin.
- Bind with basic portions of the cytoplasm.

b- Differential stain

- Using more than One dye.
- e.g.: gram stain, acid fast stain.
- Used to differentiate between 2 types of bacteria.

c- Negative stain

Stain every thing except the structure you want to visualize.

e.g.: capsule stain

Shapes of bacteria

- Cocci

Spherical shape e.g.: *Staphylococcus aureus*

- Spiral

Spiral shape e.g.: Spirochetes

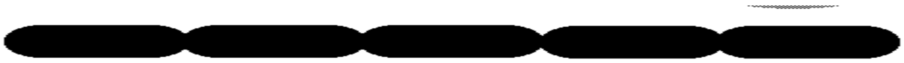
- Bacilli

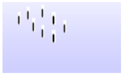
Rod shape e.g.: *Bacillus anthracis*

Arrangement

A- Arrangement of Bacilli

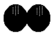
1- Bacilli chains e.g. Bacillus



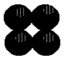
2) Scattered rods e.g. *Escherichia coli* (*E. coli*) 

B- Arrangement of Cocci

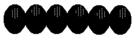
1) Monococci

2) Diplococci 

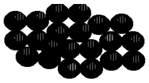
e.g.: *Neisseria gonorrhoeae*

3) Tetrads: 

e,.g.: *Micrococcus luteus*

4) Streptococci 

e.g.: Streptococcus spp.

5) Staphylococci (grape like structure): 

e,.g.: *Staphylococcus aureus*

Examination of bacteria using simple stain

1) Preparation of microscope for examination (cleaning, adjusting illumination and use oil immersion lens).
2) Preparation of a smear:

a) Using sterile loop (sterilized by heating in the flame till redness), aseptically transfer 10 loopfuls from bacterial suspension after shaking into a clean slide.

b) Dry the slide in the flame at a distance of 15 cm till complete dryness followed by passing the slide three time quickly in the flame for complete drying (heat fixation).

3) Staining by adding 2 drops of crystal violet or safranin on the bacterial film, leave for 1 min, then wash with slow stream of water.

4) Drying till complete evaporation of water.

5) Add two drops of cedar oil on the bacterial film.

6) Now you can examine the slide using oil immersion lens.

Scheme

- **Type of stain**: Simple stain
- **Name of the dye**: crystal violet, safranin.
- **Shape**: Cocci, Bacilli.
- **Arrangement**: Staphylococci, Bacilli chains and scattered rods.
- **Color**: red (pink), violet.
- **Culture**: single
- **Comment**: since crystal violet or safranin are basic dyes, then they form ionic bond with the COOH groups of the amino acids present in the cell wall leading to staining of the bacterial cell.

Exercise 3.1

1-Scheme for simple stain

2- Drawing

3- Comment

4- Identification: Unkown is

Exercise 3.2

1-Scheme for simple stain

2- Drawing

3- Comment

4- Identification: Unkown is

Exercise 3.3

1-Scheme for simple stain

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2- Drawing

3- Comment

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4- Identification: Unkown is

Module 4

GRAM-POSITIVE BACTERIA STAIN (STAPHYLOCOCCUS, SARCINA AND BACILLUS).

Differential stain

- It is used to differentiate between bacteria according their response to the stain

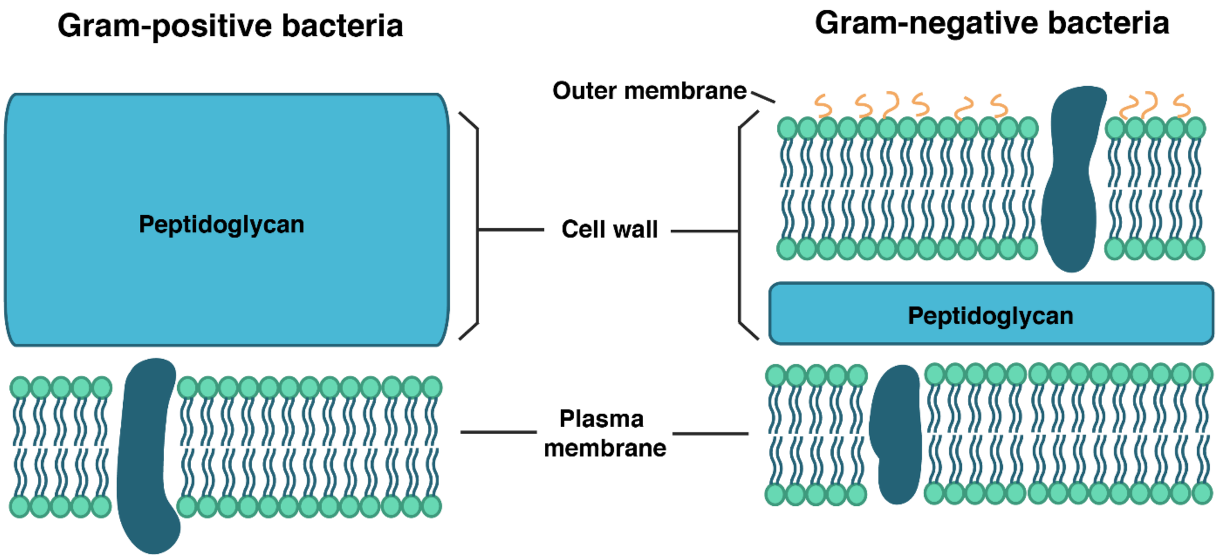
The most commonly used differential stain are

- Gram stain
- The Acid fast stain

1-Gram stain

- The Gram stain procedure was developed by Christian Gram.
- Bacteria are classified according to Gram stain into Gram positive and Gram negative bacteria.

Classification of bacteria according to the structure of cell wall "Gram-positive" & "Gram-negative" bacteria



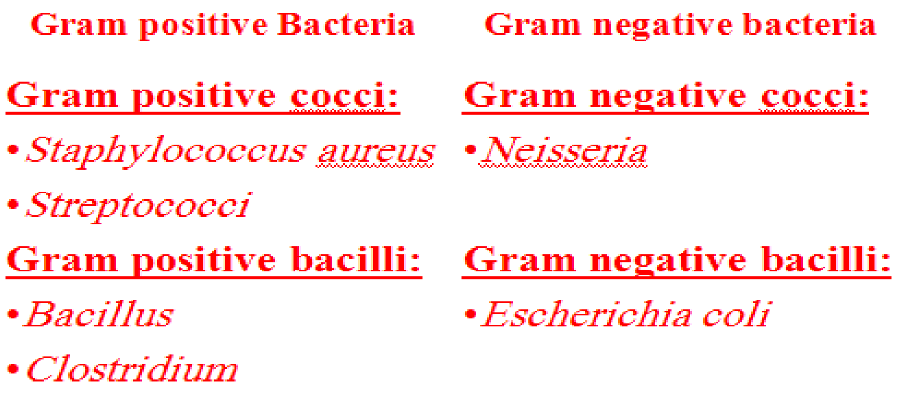
The cell wall of Gram positive bacteria and Gram negative bacteria.

Applications of Gram stain

It is very important in diagnosis and treatment of bacterial infections.

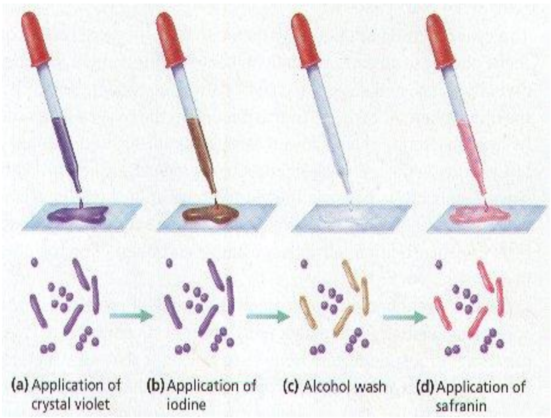
- Disease caused Gram positive bacteria: Sore through, Rheumatic fever, anthrax, botulism.
- Diseases caused by Gram negative bacteria: typhoid fever, Cholera, whooping cough, Gonorrhea.

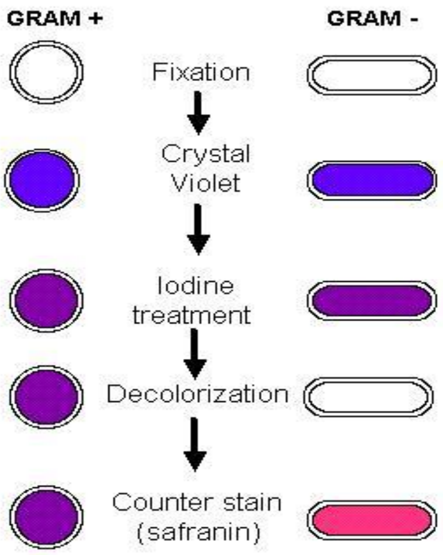
Examples Gram positive and Gram negative bacteria



Gram positive Bacteria	**Gram negative bacteria**
Gram positive cocci:	**Gram negative cocci:**
• *Staphylococcus aureus*	• *Neisseria*
• *Streptococci*	
Gram positive bacilli:	**Gram negative bacilli:**
• *Bacillus*	• *Escherichia coli*
• *Clostridium*	

Principle of Gram stain


(a) Application of crystal violet
(b) Application of iodine
(c) Alcohol wash
(d) Application of safranin



- Gram stain is used to differentiate between Gram positive and Gram negative bacteria.
- Upon staining with Crystal violet (primary stain), both Gram positive and Gram negative bacteria will take the violet color of crystal violet.
- Iodine (mordant) will form insoluble crystal violet iodine complex and both bacteria becomes deep violet.
- After decolorization with Alcohol (95% ethanol), the Gram positive bacteria will retain the deep violet color, however, the Gram negative bacteria will become colorless.
    - This is because the cell wall of Gram negative bacteria has high lipid content than Gram positive bacteria.
    - Alcohol will dissolve the lipids of the cell wall causing perforation of the cell wall with large pore size in Gram negative bacteria. So, Gram negative bacteria will easily loss the deep violet color and become colorless.
- The Gram negative bacteria will take the red color of the counter stain safranin.

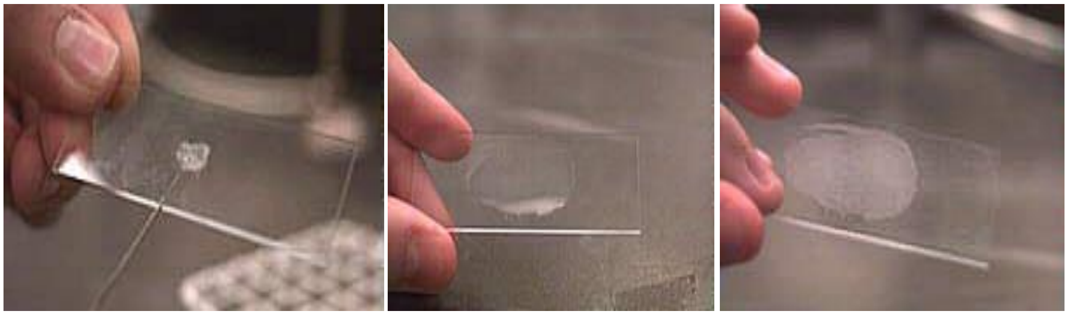
Materials:

1. Crystal violet (primary stain)
2. Iodine (mordant)
3. Alcohol (95% ethanol), Decolorizer
4. Safranin (counter stain)

Staining procedure

I- **Preparation of bacterial film.**

- Place 4-6 loopfuls of bacterial suspension in the center of a clean slide.
- Spread the suspension
- Dry and fix the bacterial film



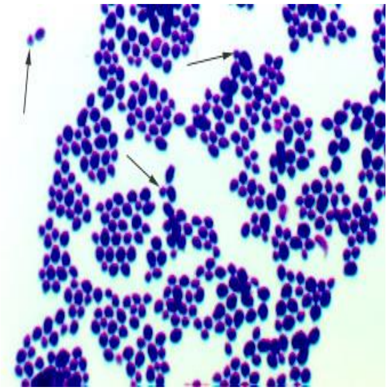
II- **Procedure:**

1. Flood the slide with crystal (or gentian) violet for 30 seconds. It is known as primary stain, then wash the slide with water. (Bacteria will be stained with violet color).
2. Flood with iodine solution for 30 seconds and then wash with water. (It will form a crystal violet-iodine complex and bacteria take deep violet color).
3. Carefully add 2 drops of 95% ethanol and wash with water. Ethanol acts as decolorizer.
    - ✓ Gram negative bacteria loss deep violet color and become colorless
    - ✓ Gram positive bacteria still have its deep violet color.
4. Flood with safranin (counter stain) for 2-3 mints and wash with water. It acts as. The colorless Gram negative bacteria will take the red color of safranin and stained with red color.

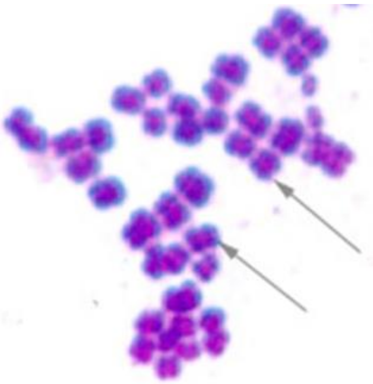
- Dry the slide and examine it under microscope.

III- Results

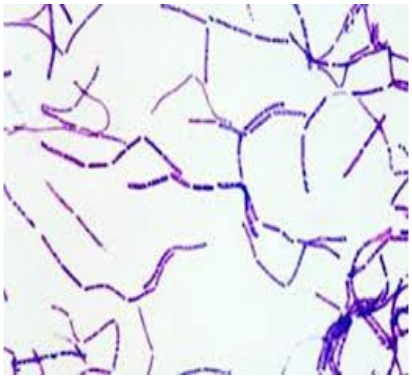
Gram-positive is stained with deep violet color.



S. aureus **stained by Gram stain**



Sarcina stained by Gram stain



Bacillus stained by Gram stain

Scheme for Gram stain

- Type of stain: Gram stain

- Name of dye: Crystal violet (1ry stain), Iodine (mordant), Ethanol 95% (decolorizer) and Safranin (counter stain)
- Color: Gram positive stained with deep violet
- Culture: Mixed
- Shape: Cocci and bacilli
- Arrangement: Grape like structure (Staphylococcus aureus)
- Comment: Due to differences in the thickness of a peptidoglycan layer in the cell membrane between Gram positive and Gram negative bacteria, Gram positive bacteria (with a thicker peptidoglycan layer) retain crystal violet stain during the decolorization process, while Gram negative bacteria lose the crystal violet stain and are instead stained by the safranin in the final staining process. A decolorizer such as ethyl alcohol or acetone is added to the sample, which dehydrates the peptidoglycan layer, shrinking and tightening it. The large crystal violet-iodine complex is not able to penetrate this tightened peptidoglycan layer, and is thus trapped in the cell in Gram positive bacteria.

Variable Gram stain

- Over decolorization:

The Gram positive bacteria will stain with red color, due to:

  - Thin film
  - More time in contact with Alcohol.
  - Excess drying
  - Conc of the stain
  - Age of culture.

Exercise 4.1

1-Scheme for Gram stain

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2- Drawing

3- Comment

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4- Identification: Unkown is

Exercise 4.2

1-Scheme for Gram stain

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2- Drawing

3- Comment

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4- Identification: Unkown is

Module 5

GRAM-NEGATIVE BACTERIA STAIN (PSEUDOMONAS, E. COLI, NEISSERIA).

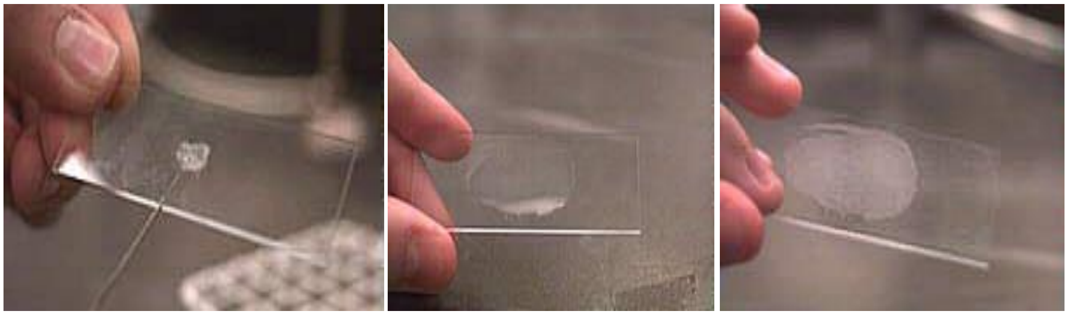
Materials:

1. Crystal violet (primary stain)
2. Iodine (mordant)
3. Alcohol (95% ethanol), Decolorizer
4. Safranin (counter stain)

Staining procedure

I- **Preparation of bacterial film.**

- Place 4-6 loopfuls of bacterial suspension in the center of a clean slide.
- Spread the suspension
- Dry and fix the bacterial film



II- **Procedure:**

3. Flood the slide with crystal (or gentian) violet for 30 seconds. It is known as primary stain, then wash the slide with water. (Bacteria will be stained with violet color).
4. Flood with iodine solution for 30 seconds and then wash with water. (It will form a crystal violet-iodine complex and bacteria take deep violet color).
4. Carefully add 2 drops of 95% ethanol and wash with water. Ethanol acts as decolorizer.
    - ✓ Gram negative bacteria loss deep violet color and become colorless

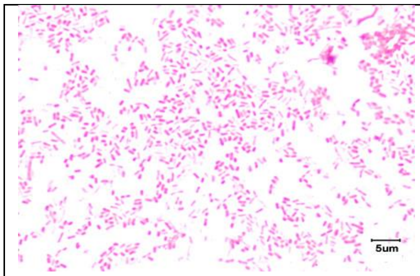
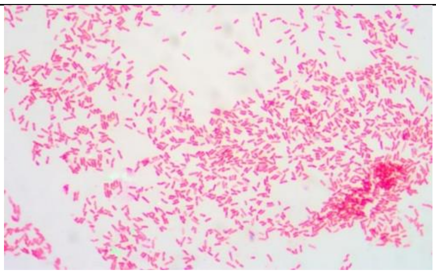
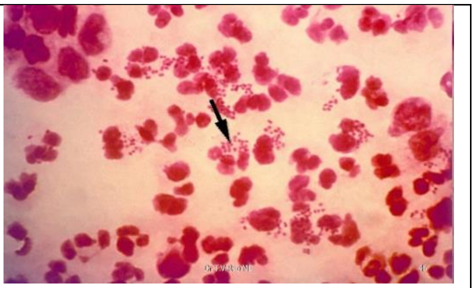
  - ✓ Gram positive bacteria still have its deep violet color.

5. Flood with safranin (counter stain) for 2-3 mints and wash with water. It acts as. The colorless Gram negative bacteria will take the red color of safranin and stained with red color.

- Dry the slide and examine it under microscope.

III- Results

Gram-negative bacteria are stained with red color.

		
Pseudomonas stained by Gram stain	*E.coli* stained by Gram stain	Neisseria stained by Gram stain

Scheme for Gram stain

- **Type of stain**: Gram stain
- **Name of dye**: Crystal violet (1ry stain), Iodine (mordant), Ethanol 95% (decolorizer) and Safranin (counter stain)
- **Color**: Gram Negative bacteria stained with red.
- **Culture**: Mixed
- **Shape**: Cocci and bacilli
- **Arrangement**: Scattered small rods (E. coli).
- **Comment**: Due to differences in the thickness of a peptidoglycan layer in the cell membrane between Gram positive and Gram negative bacteria, Gram positive bacteria (with a thicker peptidoglycan layer) retain crystal violet stain during the decolorization process, while Gram negative bacteria lose the crystal violet stain and are instead stained by the safranin in the final staining process. A decolorizer such as ethyl alcohol or acetone is added to the sample, which dehydrates the peptidoglycan layer, shrinking and tightening it. the outer membrane of Gram negative bacteria is degraded and the thinner peptidoglycan layer of Gram negative cells is unable to retain the crystal violet-iodine complex and the color is lost.

Variable Gram stain

- Under decolorization

The Gram negative bacteria will stain with deep violet color, due to thick film or less time in contact with alcohol.

Exercise 5

1-Scheme for Gram stain

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2- Drawing

3- Comment

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4- Identification: Unkown is

Module 6

MIXTURE OF GRAM-POSITIVE AND GRAM-NEGATIVE BACTERIA STAIN

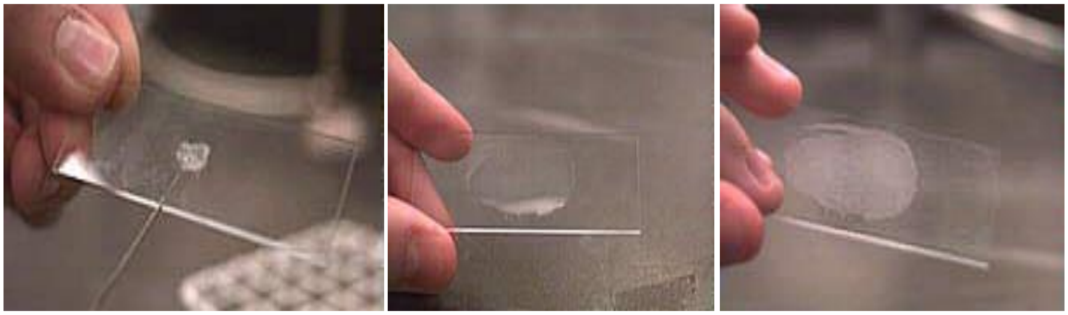
Materials:

5. Crystal violet (primary stain)
6. Iodine (mordant)
7. Alcohol (95% ethanol), Decolorizer
8. Safranin (counter stain)

Staining procedure

I- **Preparation of bacterial film.**

- Place 4-6 loopfuls of bacterial suspension in the center of a clean slide.
- Spread the suspension
- Dry and fix the bacterial film



II**- Procedure:**

5. Flood the slide with crystal (or gentian) violet for 30 seconds. It is known as primary stain, then wash the slide with water. (Bacteria will be stained with violet color).
6. Flood with iodine solution for 30 seconds and then wash with water. (It will form a crystal violet-iodine complex and bacteria take deep violet color).
5. Carefully add 2 drops of 95% ethanol and wash with water. Ethanol acts as decolorizer.
    - ✓ Gram negative bacteria loss deep violet color and become colorless

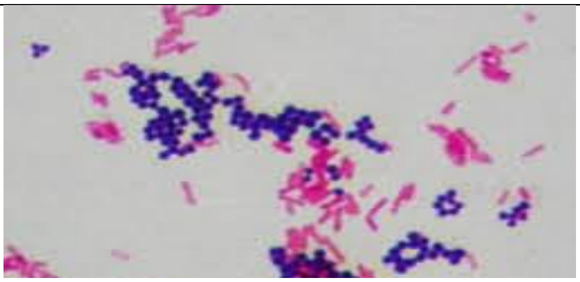
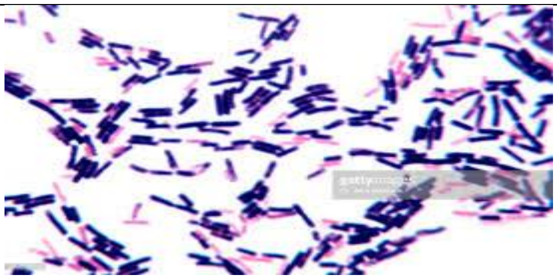
    - ✓ Gram positive bacteria still have its deep violet color.

6. Flood with safranin (counter stain) for 2-3 mints and wash with water. It acts as. The colorless Gram negative bacteria will take the red color of safranin and stained with red color.

- Dry the slide and examine it under microscope.

III- Results

– Gram-positive is stained with deep violet color.
– Gram-negative bacteria are stained with red color

	
Mixture of G+ve *S. aureus* and G-ve *E.coli* stained by Gram stain	**Mixture of G+ve Bacillus and G-ve *E.coli* stained by Gram stain**

Scheme for Gram stain

- **Type of stain**: Gram stain
- **Name of dye**: Crystal violet (1ry stain), Iodine (mordant), Ethanol 95% (decolorizer) and Safranin (counter stain)
- **Color**: Gram Negative bacteria stained with red.
- **Culture**: Mixed
- **Shape**: Cocci and bacilli
- **Arrangement**: Grape like structure (Staphylococcus aureus) and Scattered small rods (E. coli).
- **Comment**: Due to differences in the thickness of a peptidoglycan layer in the cell membrane between Gram positive and Gram negative bacteria, Gram positive bacteria (with a thicker peptidoglycan layer) retain crystal violet stain during the decolorization process, while Gram negative bacteria lose the crystal violet stain and are instead stained by the safranin in the final staining process. A decolorizer such as ethyl alcohol or acetone is added to the sample, which dehydrates the

peptidoglycan layer, shrinking and tightening it. the outer membrane of Gram negative bacteria is degraded and the thinner peptidoglycan layer of Gram negative cells is unable to retain the crystal violet-iodine complex and the color is lost. The large crystal violet-iodine complex is not able to penetrate this tightened peptidoglycan layer, and is thus trapped in the cell in Gram positive bacteria.

Exercise 6.1

1-Scheme for Gram stain

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2- Drawing

3- Comment

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4- Identification: Unkown is

Exercise 6.2

1-Scheme for Gram stain

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2- Drawing

3- Comment

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4- Identification: Unkown is

Exercise 6.3

1-Scheme for Gram stain

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4- Identification: Unkown is

Module 7

IDENTIFICATION OF CANDIDA ALBICANS

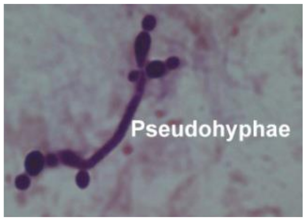
Candida Albicans is a dimorphic opportunistic fungus that grows both as yeast and filamentous cells that is the cause of many undesirable symptoms ranging from fatigue and weight gain, to joint pain and gas.

The *Candida Albicans* yeast is a part of the gut flora, a group of microorganisms that live in your mouth and intestine. When the Candida Albicans population starts getting out of control it weakens the intestinal wall, penetrating through into the bloodstream and releasing its toxic byproducts throughout the body.

Laboratory Identification

1-Gram stain

candidal hyphae and yeasts appear dark blue



2-Germ tube

Germ Tube Test is a screening test which is used to differentiate *Candida albicans* from other yeast. Germ tube (GT) formation was first reported by Reynolds and Braude in 1956. When Candida is grown in human or sheep serum at 37°C for 3 hours, they forms a germ tubes, which can be detected with a wet KOH films as filamentous outgrowth extending from yeast cells. It is positive for *Candida albicans*and *Candida dubliniensis*. Approximately 95 – 97% of *Candida albicans* isolated develop germ tubes when incubated in a proteinaceous media.

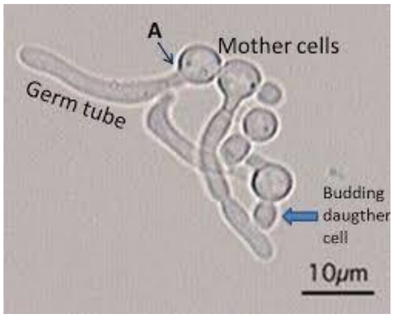
Principle

Formation of germ tube is associated with increased synthesis of protein and ribonucleic acid. Germ Tube solutions contains tryptic soy broth and fetal bovine serum, essential nutrients for protein synthesis. It is lyophilized for stability. Germ tube is one of the virulence factors of *Candida albicans*.This is a rapid test for the presumptive identification of *C. albicans.*

Procedure

1. Put 0.5 ml of sheep or human serum into a small tube. *Note: Fetal bovine serum can also be used instead of human serum.*
2. Using a Pasteur pipette, touch a colony of yeast and gently emulsify it in the serum. *Note: Too large of an inoculum will inhibit germ tube formation.*
3. Incubated the tube at 37°C for 2 to 4 hours.
4. Transfer a drop of the serum to a slide for examination.
5. Coverslip and examine microscopically under low and high power objectives.

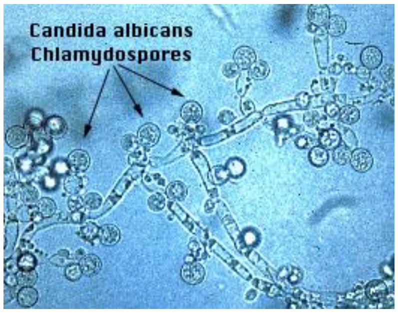
Positive Test: A short hyphal (filamentous) extension arising laterally from a yeast cell, with no constriction at the point of origin. Germ tube is half the width and 3 to 4 times the length of the yeast cell and there is no presence of nucleus. **Examples:** *Candida albicans* and *Candida dubliniensis*



Negative Test: No hyphal (filamentous) extension arising from a yeast cell or a short hyphal extension constricted at the point of origin. **Examples:** *C. tropicalis, C. glabrata* and other yeasts.

3-Chlamydospore production

Only the germ tube-positive samples were preceded for further study and to compare the rapid production of chlamydospore with corn milk broth + 5% milk. Very small inoculums were picked up with a sterile inoculating loop and was suspended in same manner as it was done for Germ tube test. The two different test tubes containing corn meal broth + 5% milk and serum milk were placed in water bath at 45°C and results were read at 8 and 16 hours, respectively.



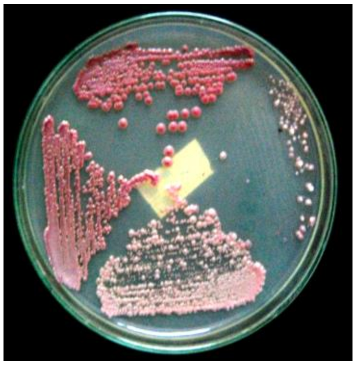
Chlamydospores were observed under wet mounts. A drop of inoculated broth media was placed onto the slide, over it a drop of lactophenol cotton blue stain (LPCB) was added then a cover slip was put over it. This was first examined under a low power objective to locate the group of cells and later, the presence of chlamydospores were confirmed and quantified in by two observers in 10 fields (high power view) in both media.

4-Sabaraud's dextrose agar (SDA)

It permit growth of *Candida*, suppresses the growth of many species of bacteria due to its low pH. Incorporation of antibiotics Chloramphenicol, gentamicin and/or tetracycline) into SDA will further increase its selectivity and cycloheximide (actidione) makes the medium selective. Antibiotics prevent the growth of bacteria, cycloheximide avoids saprotrophic fungal contamination. Typically SDA is incubated aerobically at 37°C for 24–8 hrs. *Candida* develops as cream, smooth, pasty convex colonies on SDA.

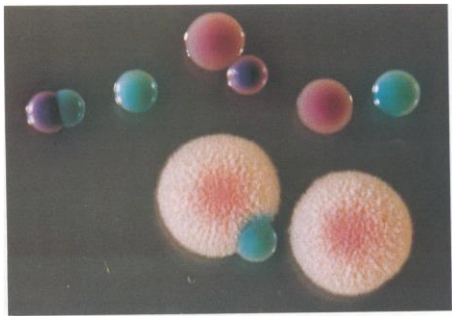
5-Pagano-Levin agar

It distinguishes between *Candida* species based on reduction of triphenyltetrazolium chloride. The medium produces pale-coloured colonies of *C. albicans,* whilst colonies of other *Candida* species exhibit varying degrees of pink coloration. Pagano-Levin agar has a similar sensitivity to SDA but is superior for the detection of more than one species in the sample.



6-CHROM agar or Brilliance Candida Agar

These media contains chromogenic substrates that react with enzymes secreted by yeast cells, resulting in various pigmentations. These enzymes are species specific and allow species identification on the basis of colony color and characteristics.



Brilliance Candida Agar incorporates two chromogens that indicate the presence of the target enzymes:

- X-NAG (5-bromo-4-chloro-3-indolyl N acetyl ß-D-glucosaminide) detects the activity of hexosaminidase.
- BCIP (5-bromo-6-chloro-3-indolyl phosphate p-toluidine salt) detects alkaline

phosphatase activity.

Chromogen: **Enzyme:**	**X-NAG** **Hexosaminidase**	**BCIP** **Alkaline phosphatase**	**Typical colony appearance**
C. tropicalis	+		bluish-purple
C. albican	+		Green
C. krusei		+	pale pink, rough colonies
C. glabrata		Variable	pink

7-Carbon and hydrogen assimilation

A carbon auxanogram was used to evaluate the capacity to assimilate a different carbon source. Yeast suspension was incorporated into a synthetic base free of carbon and solidified. After that, 16 different carbohydrates were added to the plate, which was then incubated at 35°C for 24 and 48 hours. Any visible growth, indicated by turbidity on the spot of carbohydrate incorporation was defined as constituting positive assimilation.

Exercise 7

Complete the following table with the appropriate results.

Name of the test	*C. tropicalis*	*C. tropicalis*	*C. tropicalis*	*C. tropicalis*
1-Gram stain				
2-Germ tube				
3-Chlamydospore production				
4-Sabaraud's dextrose agar (SDA)				
5-Pagano-Levin agar				
6-CHROM agar				

Module 8

ZIEHL-NEELSEN STAIN FOR ACID-FAST MICROORGANISM (T. B.)

Generally, acid – fastness is very rare among bacterial cells. Most genera are not acid-fast, with the exception of the genus Mycobacterium and some species of the genus Nocardia.

The 2 best – known acid – fast human pathogens are:

a- *Mycobacterium tuberculosis* → T.B.
b- *Mycobacterium leprae* → leprosy.

Organisms of the genus mycobacterium have a high fat content (relatively large amount of lipids) including fatty acid waxes and complex lipids. They have wax-like cell walls that are relatively impermeable.

Introduction:

Bacteria belonging to such genera as Mycobacterium have cell walls containing a waxy material called mycolic acid. This material significantly affects the staining properties of these bacteria and prevents them from being stained by many routine techniques used in the lab.

For this reason, the acid-fast stain is an important diagnostic tool in the identification of these organisms, specifically *Mycobacterium tuberculosis*, the causative agent of tuberculosis, and *Mycobacterium leprae*, the bacterium that causes leprosy.

To visualize these bacteria, it is necessary to use techniques that make the cells more permeable to stain using the Ziehl-Neelsen staining method, where:

1- The primary stain (carbolfuchsin/phenol mix) is added to cells and heated for 5 minutes. Phenol is necessary for the carbolfuchsin to penetrate the waxy cell wall and heat allows

further penetration of the stain, acting as a mordant.

2- Subsequent treatment of the cells with acid-alcohol, a decolorizer, does not remove the entrapped stain. Hence, these cells are termed acid-fast (still red in color). Cells that do not contain mycolic acid in their cell walls are easily decolorized by acidalcohol and are termed non-acid-fast (become colorless).

3- At the end of this procedure, methylene blue (counter stain) is added; the acid-fast cells will appear red to pink in color, whereas the non-acid-fast cells will appear blue.

Reagents:

1-Carbolfuchsin (primary stain).

2-Acid decolorizer.

3-Methylene blue (counter stain).

Notes:

Acid decolorizes

1- 25% H_2SO_4 → *M. tuberculosis* (as they have more lipids content i.e. more acid fast)

2- 2.5% H_2SO_4 → *M. leprae* (as they have less lipids content i.e. less acid fast).

Procedure:

1-preparation of heat fixed bacterial film.

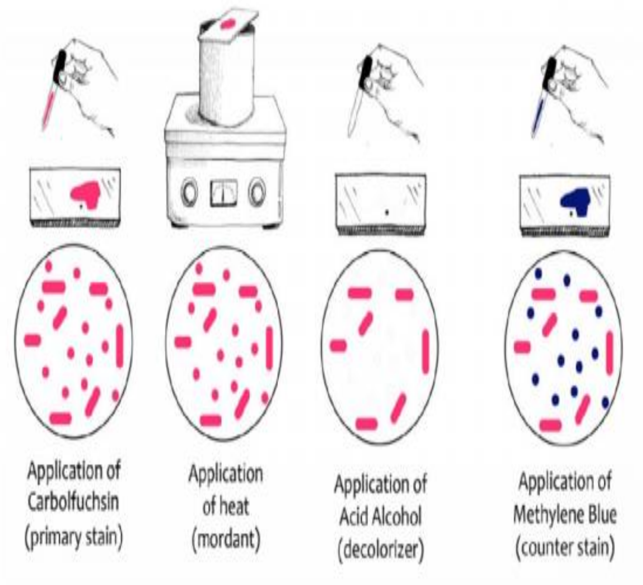
2- Staining:

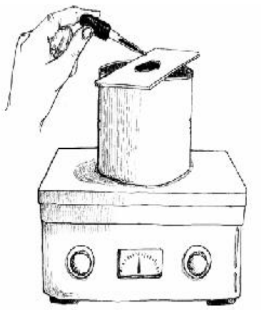
a- Flooded slide with carbolfuchsin. Heat the stain flooded slide to steaming for 5 min, allow the cool then wash excess stain.

b- Flood the slide with the decolorizer for 1 min, wash the excess.

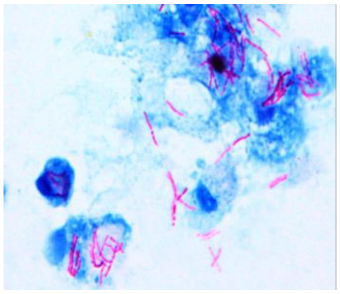
c- Flood the film with methylene blue. Allow the stain to react for 1 min, wash excess dye.

3-Dry the slide put one drop of cedar wood oil on the bacterial film and examine under microscope using oil immersion lens.


Application of Carbolfuchsin (primary stain)
Application of heat (mordant)
Application of Acid Alcohol (decolorizer)
Application of Methylene Blue (counter stain)



Results



Mycobacterium tuberculosis **stained by acid fast stain**

Exercise 8

1. What is the primary stain in the Ziehl Neelsen acid fast stain procedure?

2. Why is Mycobacterium tuberculosis easily identified by the acid fast stain?

3. What is the mordant in acid fast staining?

4. Why is heat used in acid fast staining?

5. Which bacteria was acid fast positive?

6. Why acid fast bacteria Cannot be Gram stained?

Module 9

STAINING OF SPORES (BACILLUS).

Spore formation is common among Gram positive rod shaped organisms as Bacillus and Clostridium, When they are exposed to drastic conditions during their growth as high temperature, radiation, acids, alkali or any other chemical agents such as disinfectants...ect Where the normal vegetative cells are converted in to endospores which have cell walls that can resist such drastic conditions. These walls give the spores the acid fast character (to about 0.25% H_2SO_4) When the conditions become favorable for the growth the endospores are converted to vegetative cells again.

Principle of staining:

Same as acid fast stain

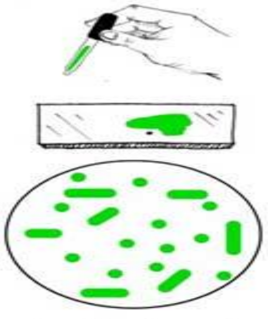
Reagents and procedures:

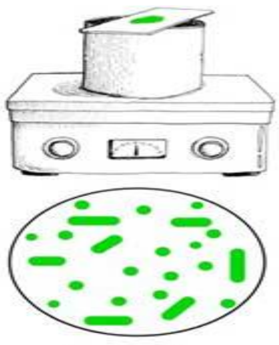
1- Same as acid fast stain but the acid decolorizer here is 0.25% H_2SO_4

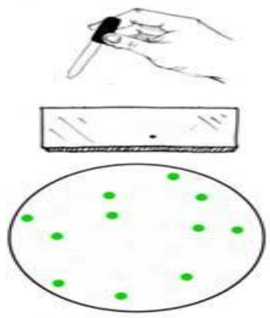
2- Other Reagents used for Endospore Staining

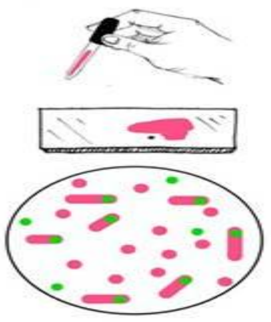
Primary Stain: Malachite green (0.5% (wt/vol) aqueous solution)
Decolorizing agent Tap water or Distilled Water
Counter Stain: Safranin

Procedure of Endospore Staining


Application of Malachite Green (primary stain)

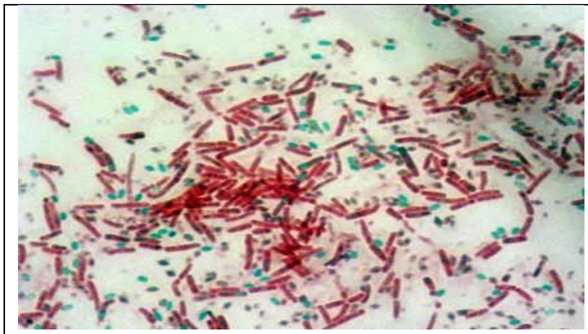
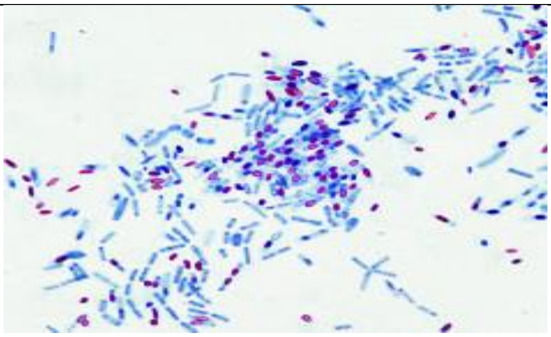

Application of heat (mordant)


Application of water (decolorizer)


Application of Safranin (counter stain)

1. Take a clean grease free slide and make smear using sterile technique.
2. Air dry and heat fix the organism on a glass slide and cover with a square of blotting paper or toweling cut to fit the slide.
3. Saturate the blotting paper with **malachite green** stain solution and steam for **5 minutes**, keeping the paper moist and adding more dye as required. Alternatively, the slide may be steamed over a container of boiling water.
4. Wash the slide in **tap water.**
5. Counterstain with **0.5% safranin** for **30 seconds**. Wash with tap water; blot dry.
6. Examine the slide under microscope for the presence of endospores. Endospores are bright green and vegetative cells are brownish red to pink.

Result of Endospore Staining

Endospores: Endospores are bright green or bright red. **Vegetative Cells:** Vegetative cells are brownish red to pink or blue.(this depend on the type of stain used)

Spores may be located in the middle of the cell, at the end of the cell, or between the end and middle of the cell. Spore shape may also be of diagnostic use. Spores may be spherical or elliptical.

Examples of Endospore Staining

Positive

Clostridium perfringens, C. botulinum, C. tetani, Bacillus anthracis, Bacillus cereus, *Desulfotomaculum* spp, *Sporolactobacillus* spp, *Sporosarcina* spp, etc.

Negative

E. coli, *Salmonella* spp, etc.

Exercise 9

1. What stains are used in an Endospore stain?

2. What would be the appearance of endospores in a simple stain?

3. What makes endospores resistant?

4. Why is being able to form endospores advantageous?

5. Why are endospores dangerous?

Module 10

WIDE DISTRIBUTION OF MICROORGANISMS, DISTRIBUTION OF MICROORGANISMS IN ENVIRONMENT.

Specific major domains consist of Archaea, Eukarya and Bacteria. Of these, the Bacteria and the Archaea are termed prokaryotes and the Eukarya are known as eukaryotes. Microorganisms within the Eukarya include fungi, protozoa and algae. In addition to the major domains, the other important biological entities are a unique group known as viruses that can infect eukaryotic or prokaryotic organisms. Viruses have no metabolic capacity and rely on infected hosts to produce viral parts that self-assemble. Similar to viruses are the viroids, which are infectious single-stranded RNAs that can infect plants. Finally, we discuss prions, which are infectious proteins that can destroy brain tissue—the transmissible spongiform encephalopathy (TSE) diseases. For all entities, structures, diversity and important environmental aspects are presented.

What are the roles of microbes in the environment?

Microorganisms are ubiquitous in the environment, where they have a variety of essential functions. Many microbes are uniquely adapted to specific environmental niches, such as those that inhabit the Dead Sea (Halobacterium), the bacteria and cyanobacteria that inhabit the boiling water springs in Yellowstone National Park, and Chlamydomonas nivalis, the algae that causes "pink snow." Microbes also play an essential role in the natural recycling of living materials. All naturally produced substances are biodegradable, which means that they can be broken down by living organisms, such as bacteria or fungi. Composting is biodegradation at its best. An examination of conditions that foster or impede composting gives insight to growth conditions of microorganisms as well as the proper function of the ecosystem.

Where Do Microbes Live?

Microbes live in almost every nook and cranny you can think of, from 20 miles beneath the Earth's surface to 20 miles overhead. They live at temperatures less than -20 degrees Celsius to temperatures hotter than the boiling point. Microbes thrive on a huge range of foods including oil and toxic wastes. Every time you walk on the ground you step on billions of microbes. Microbes live in the soil, on rocks, inside roots, buried under miles of Earth, in compost piles and toxic waste, and all over the Earth's surface. Microbes are found in boiling hot springs and on frozen snowfields. Microbes live in homes, in schools and on statues. They live on your cutting board, on your shower curtain, in your couch and in your carpet. Some microbes can survive temperatures about the boiling point. Other microbes live on snow and ice and die at room temperature. Some microbes "breathe" substances other than oxygen such as nitric acid, sulfuric acid, iron, arsenic or uranium to produce energy. Some microbes live near roots of plants in symbiotic associations. Still other microbes flourish in chemical environments harmful to humans and remove toxins like oil and pesticides.

Where there is water, there are microbes. Microbes, like other living organisms, need water to live and reproduce. However, some microbes prefer environments that have more water than the millimeters of water surrounding a particle of soil, or than is found in many foods.

Why are microorganisms important to the environment?

Environmental Benefits

Bacteria and fungi are required to maintain a healthy environment. Not only do they recycle natural wastes and dead animal and plant matter, they also produce many of the nutrients that plants need to grow. Bacteria, in particular, are the only living things that can fix nitrogen for use in plants. At the same time, microorganisms work in tandem with certain plants to aid them. Some viruses have been found to provide heat resistance to grasses in arid locations, and many plants store bacteria in their roots to help absorb certain nutrients more easily.

Microorganisms in Food

In addition to their direct environmental benefits, microorganisms are important partners when it comes to the work of creating food. They can be used to increase the fertility of the soil and increase crop yields, and they are necessary when making products like bread, beer and cheese and when growing coffee. At the same time, foods with probiotic properties, such as yogurt and certain types of chocolate, deliver helpful microorganisms to our digestive systems.

Bodily Benefits

Microorganisms known as gut flora help us digest food and regulate the production of vitamins and nutrients essential to keeping our bodies strong and healthy. Bacteria are the first line of defense the human body has against infection. The bacteria in our bodies produce natural antibiotics to repel harmful microorganisms, and if a foreign virus does infect us, many people are host to a beneficial virus that slows the rate of viral spread in the body.

Medical Benefits

We regularly aid the microorganisms in our bodies by adding more. Though certain species of microorganisms can make you sick – strep throat, the flu and measles are nothing to laugh at – modern medicine would not exist if not for the careful study of microorganisms. Bacteria and viruses are the key components of the vaccines that prevent the spread of once-deadly diseases like smallpox. Today microorganisms allow us to artificially grow helpful substances such as insulin and human growth hormones, and reprogrammed viruses are frequently used as drug-delivery mechanisms.

Technology and the Future

Applications of microorganisms in our world are constantly being studied. Certain fungi have been theorized to have anti-cancer properties, and the CRISPR Cas9 gene found in certain types of bacteria is currently being used as a gene-editing tool. Viruses have the potential to act as the future of nanotechnology, and bacteria are currently being tested as the core component of self-repairing concrete that could revolutionize infrastructure and the way we build buildings.

Protocol

1. Break up into groups of two to three students. Each group will be designing their own experiments.

2. Each group should retrieve three nutrient agar plates, two nutrient broths, two sterile saline, and four cotton swabs.

3. Each group will label a nutrient agar plate “AIR” on the bottom (NOT THE LID). Be sure to also put group initials, today’s date, and instructor’s last name (since there are multiple sections). Remove the lid from the nutrient agar plate and leave it open to the air for 30 to 60 minutes. Upon exposure of air to a sterile nutrient-agar plate or inoculation, colonies of microorganisms should appear along the surface of the agar.

4. Each group member should have one plate, one broth and two cotton swabs. The nutrient agar plate will be used to sample the environment (ex. Coke machine, washroom, or any area in the Natural and Behavioral Sciences Building). The nutrient broth will be used to sample your body or personal belongings. Label the plate and broth with location you sampled, your initials, today’s date, and instructor’s last name.

5. Using the cotton swab, inoculate the plate with a sample from the environment by swabbing back and forth all over the plate along the surface of the agar gently. Do not cut into the agar medium surface.

6. Using the cotton swab, inoculate the broth with location you sampled by placing the cotton swab into the broth and leaving it there during incubation. After incubation, the liquid media will appear turbid or cloudy.

7. Place the plates upside down and place them in your lab drawer at room temperature or in the incubator along with the broths. Placing plates upside down will prevent condensation from falling back unto the plates as they incubate.

Exercise 10

Observing Agar Plates:

1. Examine the plates and count the number of colonies on each. In the chart provided, record the number of colonies observed.

2. In the chart, describe the appearance of these colonies according to the descriptive terms for colonial morphology that were given at the end of the handout.

3. Draw sketches of colonies from each plate to illustrate colonial types.

Number and Appearance of Colonies on Nutrient Agar			
Specimen	Number of colonies on plate	Word description of colonies	Sketch of some colonies
Air			
Environment			

How are microorganisms helpful to humans?

What are used of microorganisms in food industry?

Module 11
DIFFERENT CULTURE MEDIA

The medium: It provides the nutrients and conditions needed for bacteria to grow and multiply

- Medium – what we grow bacteria in or on
  - liquid medium (media)
  - solid medium (media)

Types of the media

1. Ordinary basic media
2. Enriched media
3. Differential media
4. Media for anaerobes
5. Media for molds and fungi

I- Ordinary or basic media

It is common media suitable for cultivation of most of bacteria

It may be:

A-Fluid media

- Nutrient broth

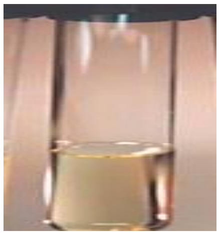
B-Solid media

- Nutrient agar

I- Ordinary or basic media

A- Fluid media

Nutrient broth:



Uses: It is a common media for cultivation of bacteria.

Composition: boiled ground meat with water, filter to get clear solution (meat extract), pepton, 0.5% NaCl, neutral PH, sterilized.

Shape: light yellow transparent fluid

Pepton water:

Composition: 1% pepton, 0.5% NaCl and water, the components are dissoved by heat, filter, sterilized

Uses: used in indol production test

Used as base of sugar media

B- Solid media

Nutrient agar:

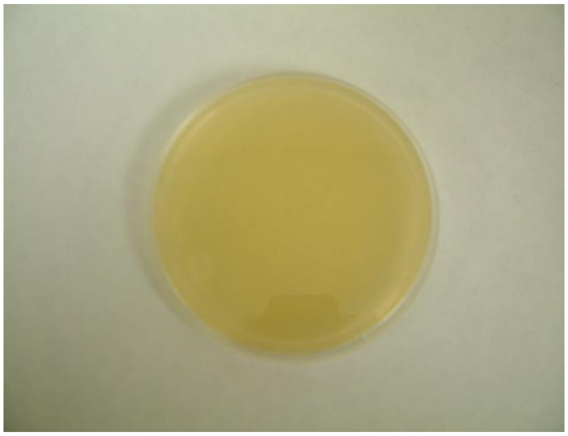
Composition

- It is composed of nutrient broth with (1.5-2%) agar.
- Solid at room temp, it liquefies by heating above 45 C

Uses: it is common media for cultivation and isolating bacteria

Forms:

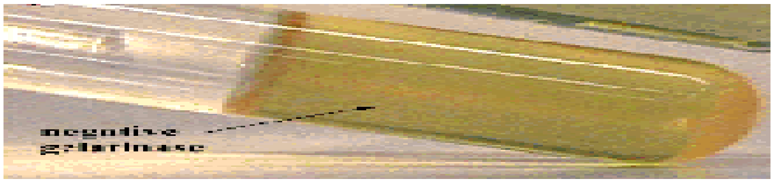
1. Agar plate: used for separation and isolation of pure colonies
2. Agar slant: used for short storage of bacteria 3-6 weeks
3. Deep agar: used for prolonged storage of bacteria 6 months



Gelatin media:

Composition: It is composed of 10-15 % gelatin in nutrient broth and sterilized in open autoclave for 20-30 mins for 3 successive days

Uses: used to test proteolytic organism (gelatin hydrolysis test)



II- Enriched media

– Contain growth factors and other complex organic substances like blood, serum, etc

- Better for pathogenic bacteria
- Types:
  - ➢ Blood agar
  - ➢ Chocolate agar
  - ➢ Lofflers serum
  - ➢ Lowenstein Jensen medium

1- Blood agar

Preparation: melt nutrient agar to 100 C, cool to 55 C, Add 5-10 % sterile blood of sheep or ox, mix well, poured into plate or slopes and allowed to cool and solidify.

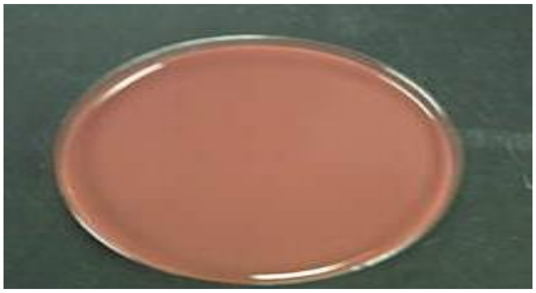
Color: red opaque color

Uses: As Enriched media

To differentiate organism according to the action on blood.

2- Chocolate agar Heated blood agar

- Preparation : it is prepared as blood agar then raise the temperature to 100 c for 2 mins then pour into plate.
- Shape: it is brown chocolate color.
- Uses: used for haemophilus group of organisms



3- Lofflers serum

Composition: ox or horse serum in glucose broth and sterilized in hot air oven at 75 C for 3 hrs on 2 successive days

Shape: opaque white media

Used for cultivation of *Corynbacterium diphteria*.

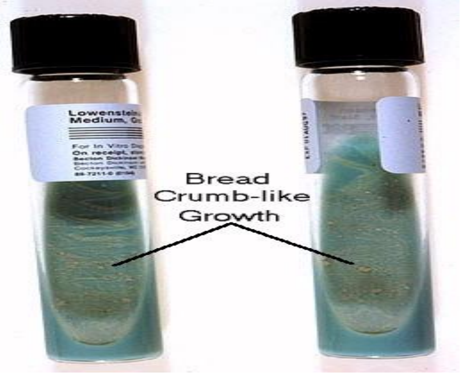
4- Lowenstein Jensen media

Composition: It is egg media contains glycerin, potato starch, Asparagine and Malachite green

Shape: opaque green media

Uses:

It is selective media for TB



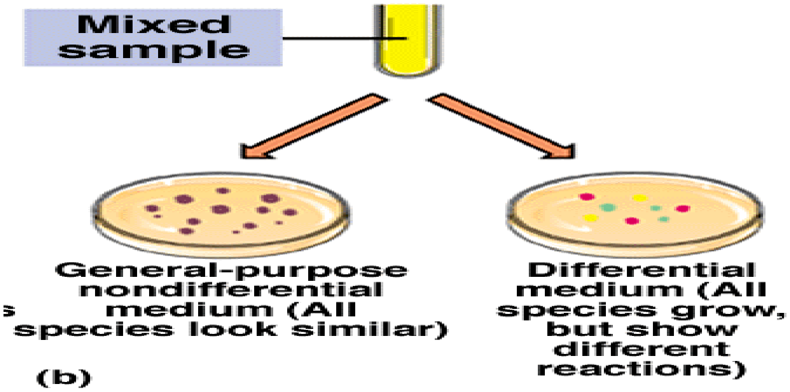
IV- Differential media

This media used to differentiate between the bacteria by change in the color of the growing colonies.

Examples:

MacConkey's media (non selective and differential)

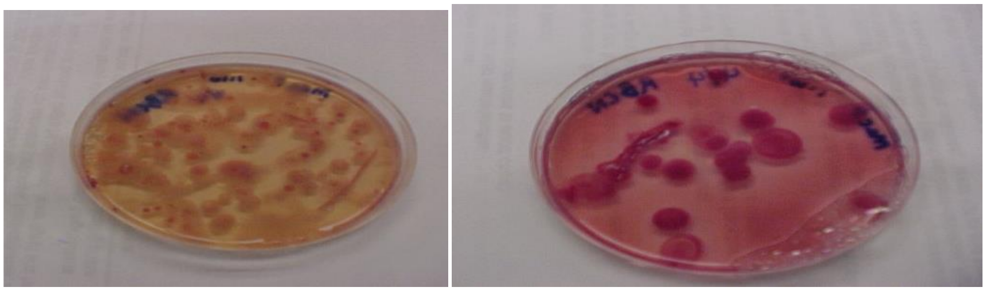
Simmon's citrate media (selective and differential)


Mixed sample
General-purpose nondifferential medium (All species look similar)
(b)
Differential medium (All species grow, but show different reactions)

MacConkey's media

This media contains lactose and acid indicator

Certain Bacteria grow on this media causing lactose fermentation with production of acid which will change the color of the indicator (neutral red) and the bacterial colony appear pink color. Example: *E. coli*
Other organisms such as Typhoides can grow on the media but not cause lactose fermentation.
So the bacteria colonies will not take the pink color



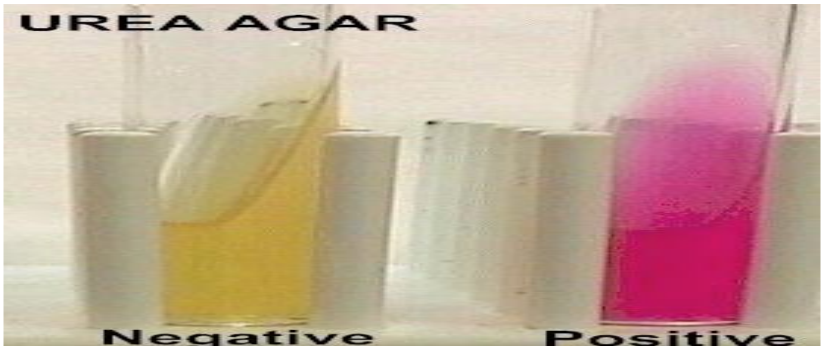
Simmon' citrate media (slant)

- This media used to test the ability of the organism to utilize citrate as a source of Carbon. This media has a pH indicator called Bromothymol Blue added that detects the change in pH. In acidic pH indicator is green and in alkaline a pH the color is blue
- Upon citrate utilization the PH of the media will be reduced causing change in color of the media into blue
- (green slant)

Christensen's urea medium

this medium used to test the ability of the organism to produce urease enzyme which split urea in urea media to form ammonia and CO2.

The accumulation of the ammonia will produce alkaline PH which turn the color of indicator (phenol red) into pink.



V-Media for anaerobic organisms

Thioglycollate media

- Thioglycollate Media are recommended for the cultivation of aerobic, facultative, and anaerobic microorganisms
- Fluid Thioglycollate Medium is used for sterility testing of biological specimens.
- The media is incubated at 30-35 C for 7 days
- Composition: small amount of agar in Thioglycollate Medium aids by decreasing the diffusion of oxygen into the medium so assist the growth of the anaerobes near the bottom. And the aerobic organism can grow near the top of the media.
- Sodium thioglycollate and cystine are a reducing agent which maintains a low oxygen.
- Presence of indicator (resazorine) on the top of media to indicate the oxygen content.

Cocked MEAT MEDIUM

Cocked meat broth is used for culturing anaerobic organisms.

VI-Media for cultivation of yeast and fungi

Fluid Sabouraud's media

- This media used for cultivation of yeast and mold

- Composition: glucose, pepton and water
- The pH is 5.6 acidic pH
- The acidic pH will favor the growth of fungi.
- It is used for sterility testing of biological specimens

Shape: yellowish brown fluid media

The media incubated at 25 c for 10 days.,

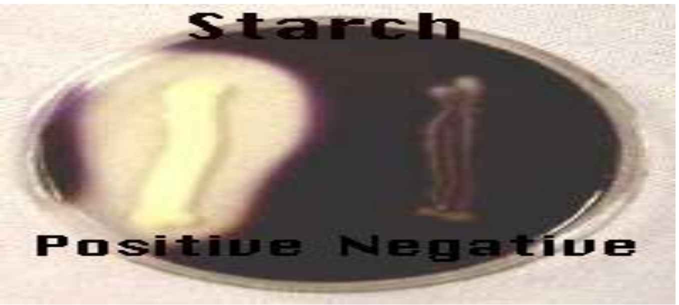
Starch hydrolysis

Objective: to test the ability of the organism to produce exoenzyme amylase which break down the starch into monosaccharide

- Starch is a complex carbohydrate of large molecule cannot pass through the cytoplasmic membrane so some organisms produce an exoenzyme amylase to hydrolysis starch into monosaccharide can used by these microorganisms

Procedure:

- Plate of starch agar inoculated by microorganism
- Upon addition of I2: amylase producing organism is surrounded by a clear zone (monosaccharide) while the remaining of the media will stain with the violet color



Casein hydrolysis

Objective: to test the ability of the organisms produce proteolytic exoenzymes (proteinase which hydrolysis casein).

Casein is the main protein of milk that is responsible for the white color of milk.

Hydrolysis of casein will form more soluble and transparent compounds (peptides and amino acids).

- The process of casein hydrolysis is called peptonization or proteolysis.
- The process of casein hydrolysis is indicated as the following :
- Upon growing the organism on casein media the area surrounding the proteinaseproducing organism will appear transparent.
- Casein hydrolysis positive organisms (A) and a negative organism (B).

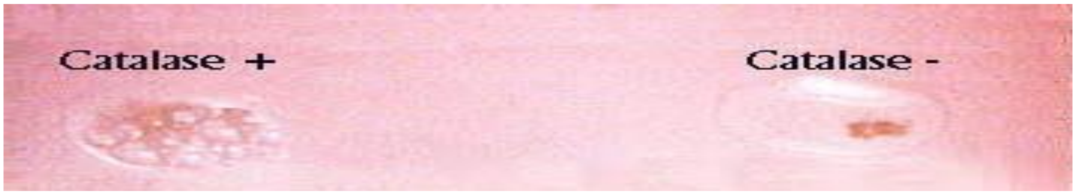
Calatase production

TO Test the ability of the organism to produce catalase production

The enzyme degradate H_2O_2 into oxygen and water with production of air bubbles.

Procedure:

Add H_2O_2 on the bacterial growth the presence of gas bubbles means that the organism produce catalase enzyme



Oxidase production

- It indicates the presence of cytochrome C in the respiratory chain.
- Aerobic microorganisms with c cytochrome can oxidize certain amines to form colored products.

MATERIALS:

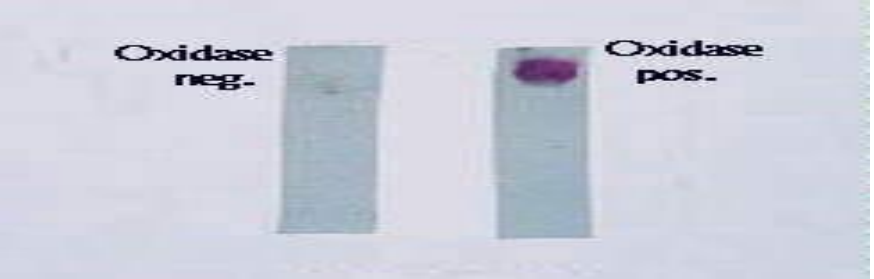
Filter paper 1% N,N,N'N' tetramethyl-p-phenylenediamine

METHODS:

Pre-wet filter paper with oxidase reagent (N,N,N',N'-p-phenylenediamine) and allow to dry.

- Pick a bacterial colony with a sterile toothpick.

Gently scratch the colony onto the filter paper. A purple color is produced.

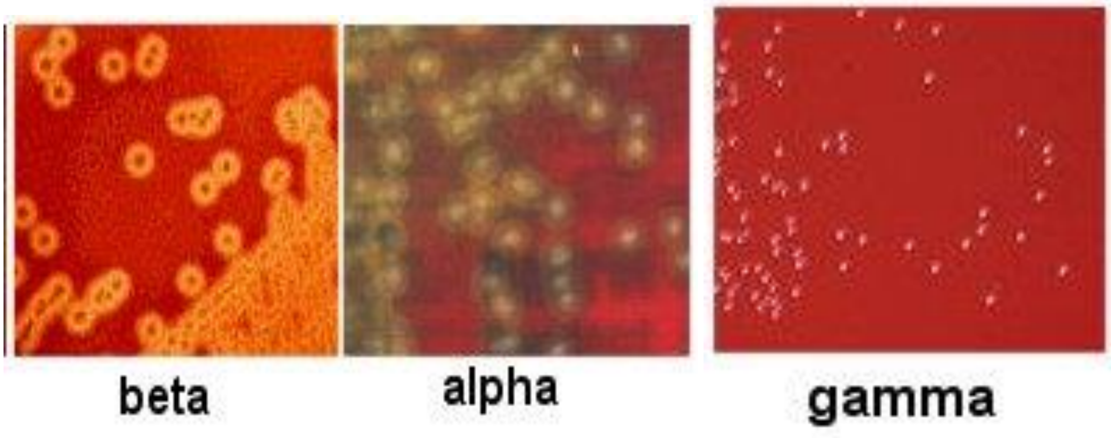


Hemolysin production

Objective:

To test the ability of the micro organism can produce exoenzymes hemolysin which has destructive effect on the blood cells

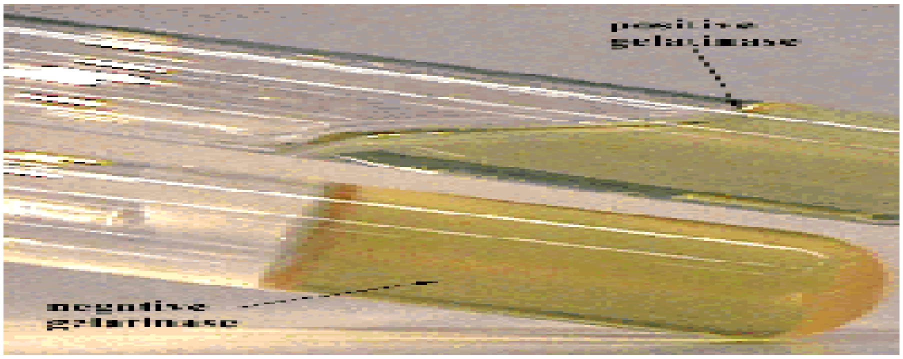
- Some microorganisms completely destroy the blood cells resulting in complete hemolysis of the surrounding area known as B-hemolysis
- Other microorganism partially hydrolysis hemoglobin resulting in greenish area known as Alpha hemolysis.
- Organisms donot produce hydrolysis and have no effect on the red blood cells are gama-hemolysis.



Gelatin hydrolysis

Objective: to test the ability of the microorganism produce exoenzyme gelatinsae which liquefy gelatin

The gelatin hydrolysis is indicated by *loss in ability to solidify even after refrigeration*

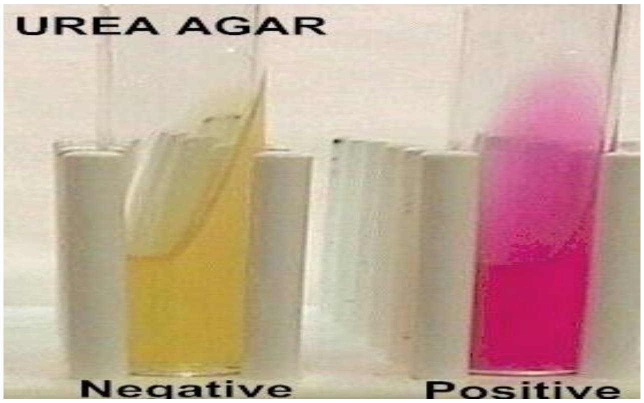


Urease production

Objective:

to test the ability of the organism to produce **urease enzyme** which split **urea in urea media** to form ammonia and CO2.

The accumulation of the ammonia will produce alkaline PH which turn the color of indicator (**phenol red)** into **pink**.

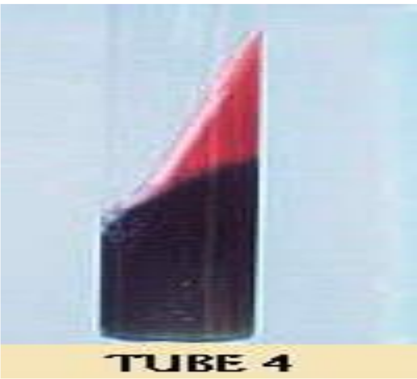


Hydrogen sulfide production

Objective:

To test the ability of the organism to use organic sulphur (cystine, cystein and methionine) or inorganic sulphur (thiosulphate, sulphite or sulphate) with the production of hydrogen sulfide, which is detected by iron salt. The presence of black precipitate is indication of H2S production Procedure:

- Inculcate media pepton iron agar or triple sugar iron (sodium thiosulfate)
- Black color will indicate H2S production (tube 4).



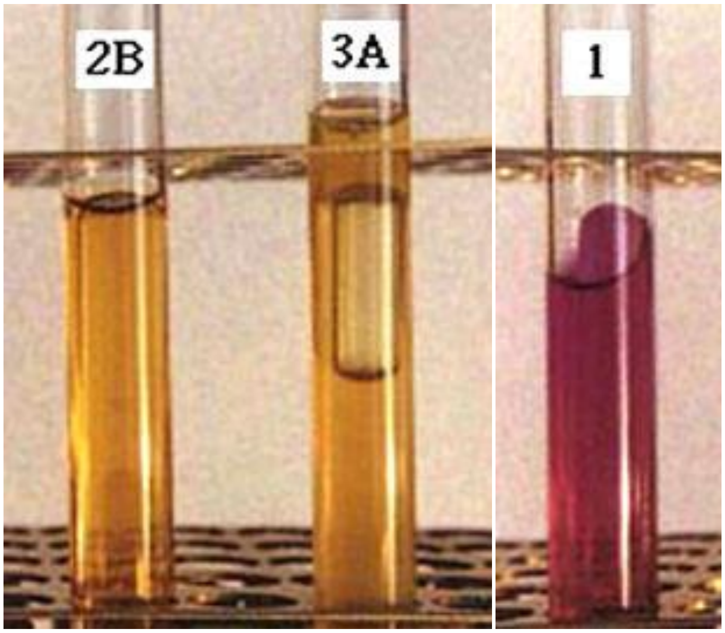
Sugar fermentation

It is used to test the ability of the organism to ferment sugar to produce acid or acid and gas.

Procedure:

- Inoculate the carbohydrate media with the organism.
- The media contains phenol red indicator (red in the alkaline media and turns yellow in the presence of acid)
- The presence of inverted Durhame's tubes can detect the gas production
- Tube 1: No fermentation. The pH indicator remains red.

- Tubes 2B: Fermentation with the production of acid (yellow color) but no gas.
- Tubes 3A : Fermentation with the production of acid (yellow color) and gas (bubble in Durham tube).



Nitrate reduction

Objective: test the ability of the organism to produce nitrate **reductase** enzyme which can reduce nitrate into nitrite

Procedure:

- Inoculate the organism into nitrate broth
- Incubate at 37 C for 48 hrs
- Add 1 ml of coupling reagent (sulfanilic acid and 1 ml of dimethyl alpha naphthyl amine reagent)

the organism produce nitrate reductase enzyme the nitrate in the media will reduced into nitrite and the color become red **precipitate.**

Ammonia production

Objective : to test the ability of the organism to degradate the organic nitrogen in the protein into ammonia.

Procedure:

- Incubate tube of 4% peptone water with organism
- Incubate at 37 c for 2, 4, 7 days
- Add **nesseler's reagent.**
- Appearance of yellow, orange or brown color indicate positive test.

IMViC

IMViC is

- Indole
- methyl red
- Voges-Proskauer
- citrate .

The IMViC tests are employed for

- identification of Enterobacteriaeae (enterics which includes such organisms as *Klebsiella*, *Enterobacter*, and *Escherichia coli*.
- The presence of *E. coli* is an indicator of fecal contamination of food and water. While *Enterobacter* and *Klebsiella* resemble *E.coli* in being lactose fermenters, their presence does not necessarily indicate fecal contamination because they are widespread in soil and grass.

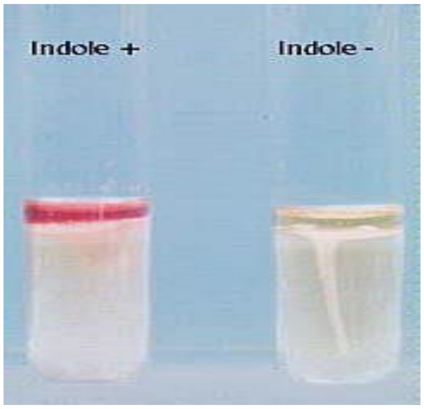
INDOLE TEST

Some bacteria have the ability to break down tryptophan into indol.

Procedure:

- Incubate tryptone broth media with the tested organism.

- the presence of indole can be detected through the use of Kovac's reagent (para dimethy aminobenzaldehyde in amyl alcohol).
- Kovac's reagent, which is yellow, reacts with indole and produces a red color on the surface of the test tube.



The Methyl Red and Voges-Proskauer's Tests

- The methyl red (MR) and Voges-Proskauer (VP) tests is done on MR-VP broth.
- MR-VP media contains glucose and peptone.

In methyl red test

- *In methyl red test the test organism can ferment the glucose and produce acid which will change the color of the Methyl red indicator into red color*
- *E. coli* is one of the bacteria that produces acids, causing the pH to drop below 4.4. When the pH indicator methyl red is added to this acidic broth it will be cherry red (a positive MR test).
- *Klebsiella* and *Enterobacter* produce neutral products from glucose (e.g. ethyl alcohol, acetyl methyl carbinol). causing the pH to rise above 6.2. At this pH, methyl red indicator is a yellow color (a negative MR test).

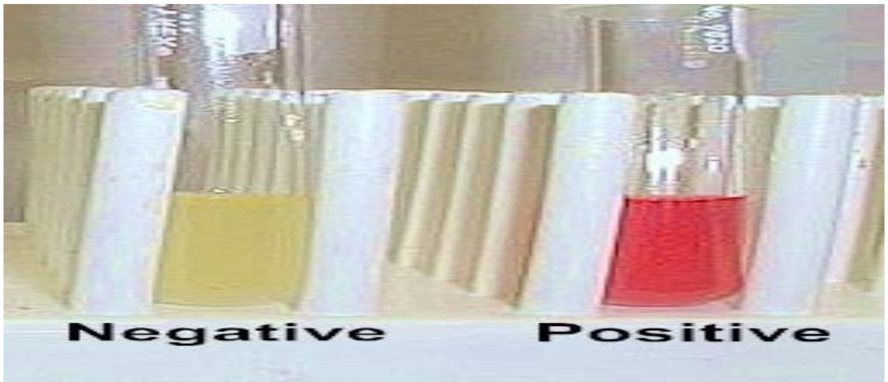
Vogues-Proskaure's test

- The reagents used for the VP test are Barritt's A (alpha-napthol) and Barritt's B (potassium hydroxide).
- When these reagents are added to a broth in which acetyl methyl carbinol is present, they turn a pink- color (a positive VP test). This color may take 20 to 30 minutes to develop.
- *E. coli* does not produce acetyl methyl carbinol, but *Enterobacter* and *Klebsiella* do.

Methyl Red-Voges Proskauer Tests

- *E. coli* is (MR+/VP-)

- Enterobacter aerogenes is (MR-/VP+).

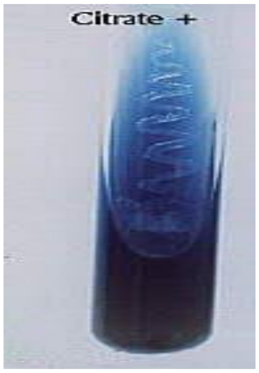


CITRATE TEST

- The citrate test is used to determine the ability of a bacterium to utilize citrate as its only source of carbon.
- Bacteria can break citrate into organic acids and carbon dioxide. The carbon dioxide can form a basic compound, sodium carbonate.
- A pH indicator Bromothymol Blue in the medium detects the presence of this compound by turning **blue** (a positive test).

Procedure

- Simmon’s Citrate media used to test the ability of the organism to utilize citrate as a source of Carbon.
- Upon citrate utilization the PH of the media will be reduced causing change in color of the media into blue
- (green slant)



III- carbohydrate media

Composition:

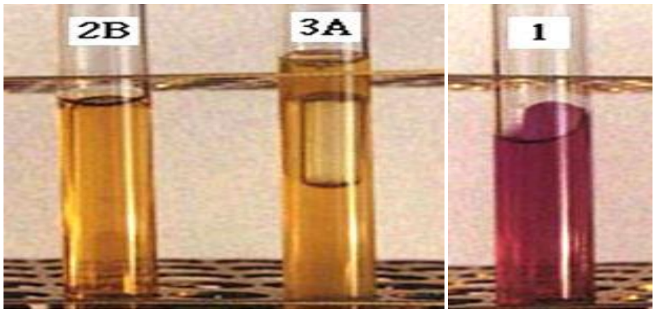
It is composed of various types of sugars added to broth media.

Sugars: glucose, mannose, galactose, maltose and lactose.

It is used to test the ability of the organism to ferment sugar to produce acid or gas or both.

- Tube 1: No fermentation. The pH indicator remains purple.
- Tubes 2B: Fermentation with the production of acid (yellow color) but no gas.

Tubes 3A : Fermentation with the production of acid (yellow color) and gas (bubble in Durham tube).



Exercise 11

State the following statements are TRUE or FALSE:

1. Streptococci grow well on Mannitol Salt Agar.
2. The chocolate within chocolate agar provides the nutrients required for bacterial growth.
3. Lowenstein-Jenson slopes contain penicillin.
4. Chococlate agar is made from blood agar.
5. Sabouraud Dextrose agar is used to cultivate bacteria.

Choose the best correct answer:

1. Which all-purpose bacterial growth medium will we be using most often in lab?

a. MacConkey's (MAC) b. Tryptic Soy Agar (TSY)
c. Mannitol Salt Agar (MSA) d. Blood Agar (BAP)

2. MacConkey's Agar will only grow…

a. halophilic bacteria b. Gram-positive bacteria
c. Gram-negative bacteria d. Staphylococcus

3. Which type of media will you be pouring into Petri dishes?

a. MacConkey's (MAC) b. Tryptic Soy Agar (TSY)
c. Mannitol Salt Agar (MSA) d. Blood Agar (BAP)

4. Mannitol Salt agar (MSA) is…

a. differential only b. selective only c. both differential and selective d. neither

5. All of the following are true about agar except it

a. liquefies at 100°C.
b. is a polysaccharide derived
c. is a polysaccharide derived from a red alga
d. Solidifies at approximately 40°C.

6. The addition of which of the following would change a chemically defined

medium into a complex medium?

a. Biotin (a vitamin)
b. K2HPO4
c. NH4NO3
d. Yeast extract

7. Addition of blood to a culture medium only allows the hemolytic bacteria that grow on the plate to be picked out. This is an example of a

a. Differential media.
b. Liquid media.
c. Complex media.
d. Selective media.

8. Which of the following is used as a solidifying agent for media?

a. Beef extract
b. Peptone
c. Agar
d. Yeast extract

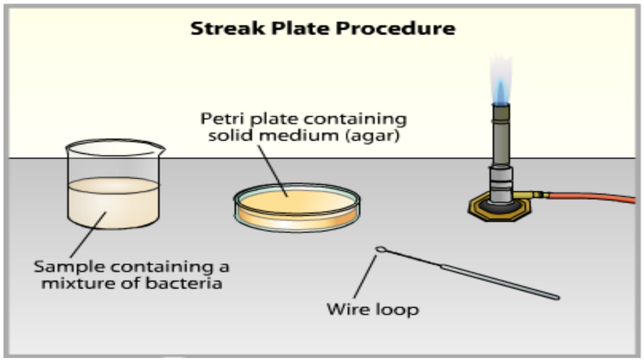
9. Which of the following is a Complex media for fungal growth?

    a. Nutrient broth
    b. Luria-Bertani media
    c. Potato Dextrose Agar(PDA) media
    d. Mac Conkey Agar media

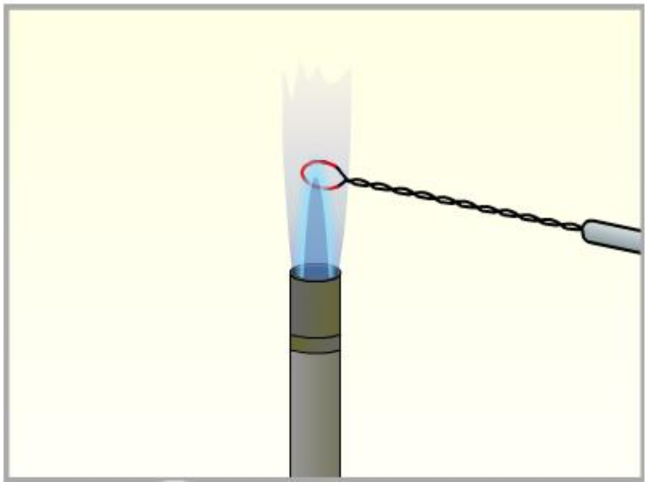
Module 12
STREAKING TECHNIQUES

There are several techniques for obtaining isolated bacterial colonies. The classical method for isolating individual strain of bacteria from a sample is called the streak plate procedure. The technique involves the following steps:

1- The student begins by sterilizing a wire loop in a flame.
2- To cool the sterilized loop, the student touches it to a sterilize agar plate. The student the dips the loop into a sample containing a mixture of bacteria. Depending on the sample, the loop may pick up hundreds or thousands of bacteria which are spread back and forth across the surface of the agar.
3- Before continuing to streak the plate, the remaining bacteria on the loop are first killed in the flame. After cooling the sterilized loop, the student drags it through the previous path, picking up a small number of bacteria and spread them into a new area of the plate.
4- After sterilizing and cooling the loop again, the procedure is repeated. With each new path, the loop picks up a small number of bacteria, and therefore can spread them farther and farther apart.
5- During the incubation, the bacteria multiply and produce colonies. Each colony consists of millions of cells derived from a single parent bacterium. The isolated colonies found in the last streak represent isolated strains. To ensure the strain purity, the streak plate procedure is usually repeated a few more times, using an isolated colony as the source of starting bacteria.


Streak Plate Procedure
Petri plate containing solid medium (agar)
Sample containing a mixture of bacteria
Wire loop

(1)



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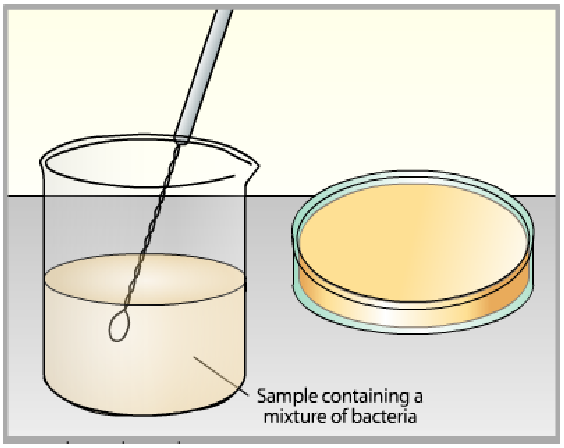
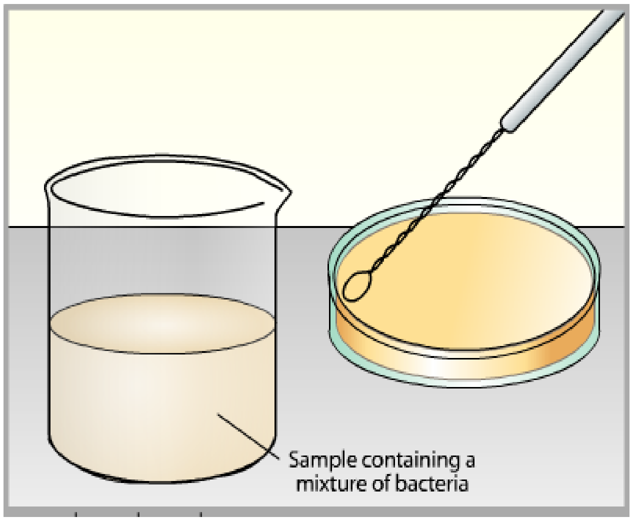
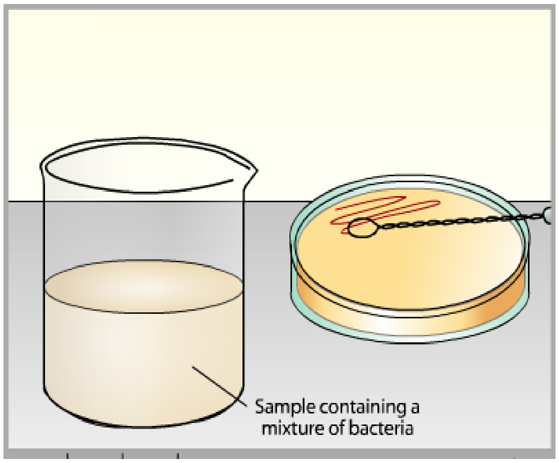

Sample containing a mixture of bacteria

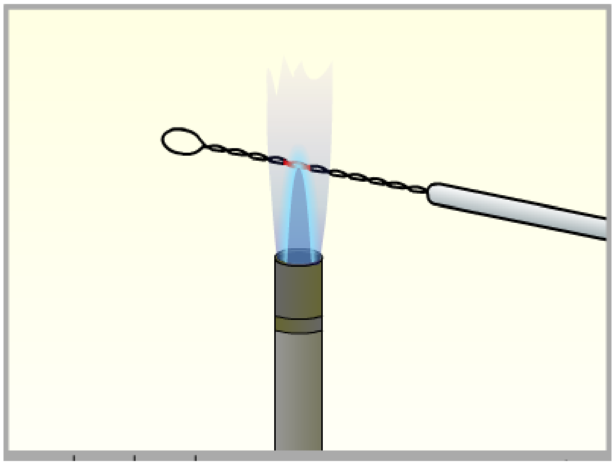
(3)


Sample containing a mixture of bacteria

(4)


Sample containing a mixture of bacteria

(5)



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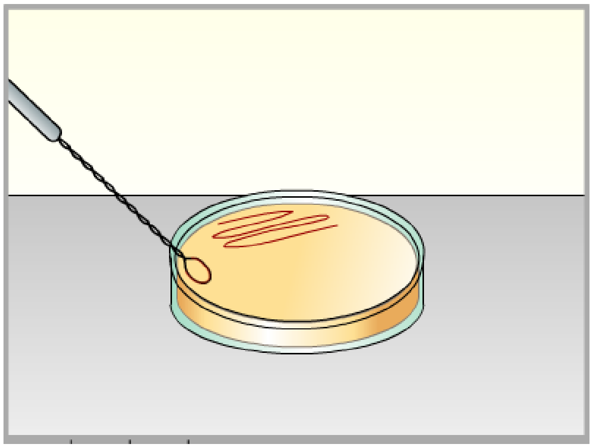

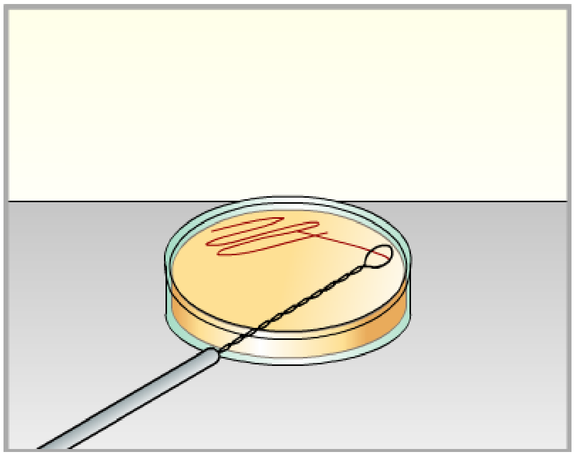

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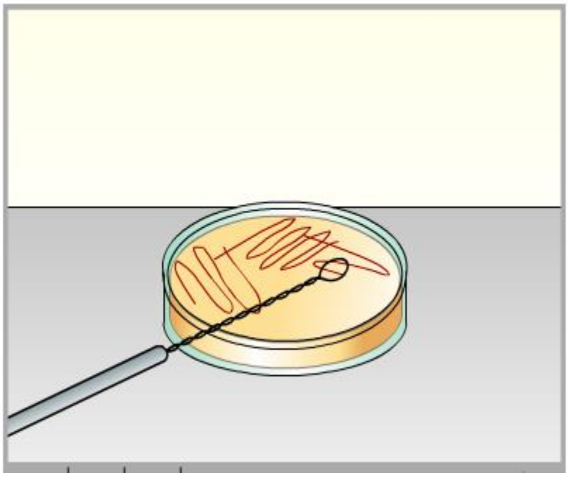

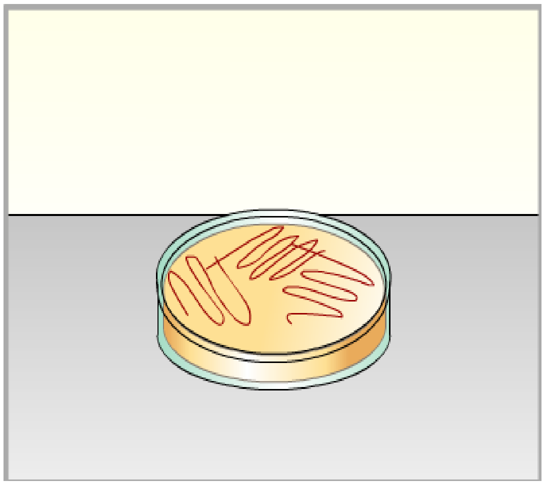

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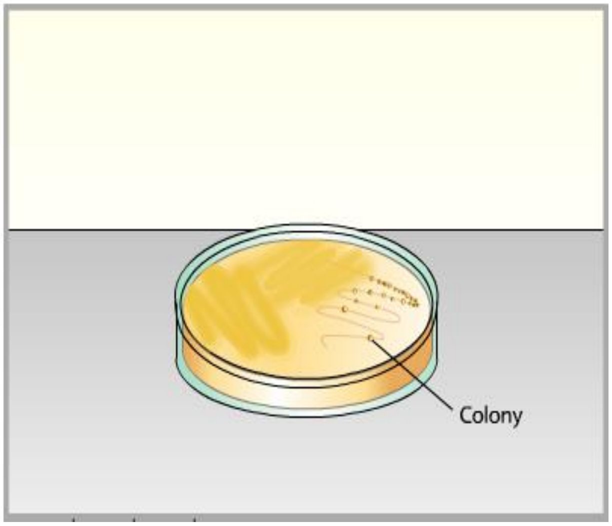

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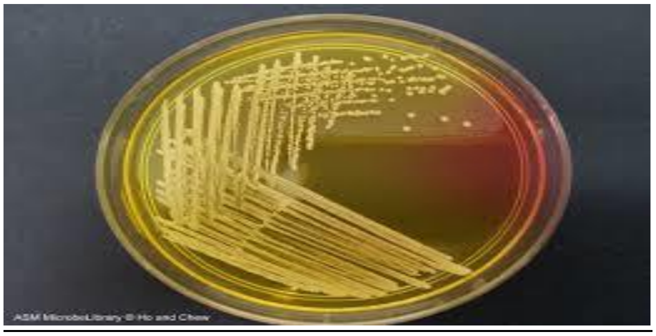


Exercise 12

Choose the best correct answer

1. Which of the following observations would suggest that a plate was inoculated with a pure (axenic) culture?
   a) Isolated colonies are all white in color and about the same size.
   b) Bacterial growth is apparent along the streaks connecting each quadrant.
   c) Bacterial growth is apparent in all four quadrants.
   d) Isolated colonies are all white in color, but some colonies are noticeably larger than others.

2. Imagine that you forgot to flame the loop before streaking the inoculum from the first quadrant into the second quadrant. What is the most likely consequence of this error?
   a) Too much bacterial growth outside the first quadrant.
   b) Contamination of the broth culture.
   c) Too little bacterial growth outside the first quadrant.
   d) Contamination of the Petri plate culture.

3. A student INCORRECTLY streaks the plate according to the diagram below, creating streaks in the order: a, b, c, d. Which of the following is a streaking error shown in the illustration?
   a) Streak d does NOT draw inoculum from streak c.
   b) Streak c does NOT draw inoculum from streak a.
   c) Streak c goes outside quadrant 3.
   d) Streak b draws inoculum from streak a

4. Which of the following images shows how to properly open a Petri plate to streak the agar?
   a) lifting the lid only enough to get the loop inside.
   b) lifting the lid completely to the left side.
   c) removing the lid and placing it on your work bench.
   d) lifting the lid and allowing it to rest on top of the inoculating loop.

5. Where should you label your Petri plate with information about the culture?
   a) On the inside of the lid.
   b) On the top of the lid.
   c) On the bottom of the plate.
   d) On the side of the bottom (agar-containing) portion of the plate.

6. A student streaks a plate using inoculum from a broth culture tube. The culture tube was labeled "Escherichia coli," indicating that the culture was composed of a single bacterial species. Which of the following best explains the unexpected plate result shown here after incubation?
   a) The plate was exposed to airborne contaminants.
   b) The loop wasn't flamed between quadrant streaks.
   c) The plate was not rotated properly during the inoculation process.
   d) The culture in the tube was contaminated.

Module 13

ISOLATION OF PURE COLONIES ON NUTRIENT AGAR AND ON BLOOD AGAR

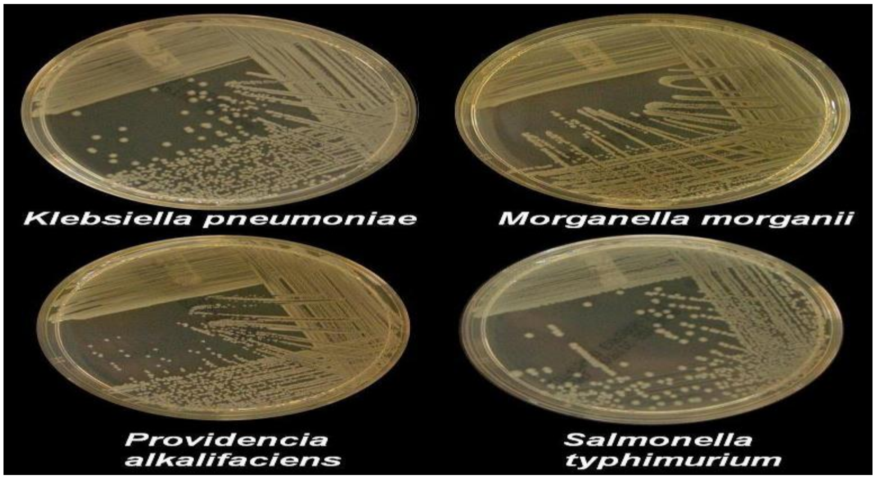
Most environments carry a mixed microbial population. To fully appreciate the contribution of each group of organisms to the ecology of the mass, one must first dissect this mixed culture to obtain single colonies. The single colony is transferred (picked) to a fresh medium to obtain a larger, homogeneous culture that may be studied and characterized by a variety of techniques. One such technique is called aseptic technique. Microbiologists and health workers use this technique to prevent contamination of cultures from outside sources and to prevent the introduction of potential disease agents into the human body (infection can occur through contamination of your hands and clothing with material from your bacterial cultures).

Aseptic Techniques

Aseptic techniques (also called sterile techniques) are defined as the processes required for transferring a culture from one vessel to another without introducing any additional organisms to the culture or contaminating the environment with the culture. The following conditions must exist for aseptic technique to be successful: 1. The work area must be wiped with an antiseptic to reduce the number of potential contaminants. 2. The transfer instruments must be sterile. 3. The work must be accomplished quickly and efficiently to minimize the time of exposure during which contamination of the culture or laboratory worker can occur.

General, Selective and Differential Media

Nutrient Agar (NA), Supplemental Nutrient Agar (SNA), Luria or Luria-Bertani agar (L or LB), Trypticase Soy Agar (TSA): These media are general cultivation and maintenance media used for many environmental organisms that do not have special growth requirements, such as E. coli, Staphylococcus sp. and Bacillus sp.



Sheep Blood Agar (SBA): This is a medium with TSA, SNA or BHI as a base and 5% sheep blood. Blood is incorporated into the medium to provide growth factors required by fastidious pathogens. Bacteria may also be characterized by their ability to cause hemolysis of the blood cells. Alpha (α) hemolytic bacteria produce a zone of partial clearing (greening) around single isolated colonies; beta (β) hemolytic bacteria produce a complete zone of clearing surrounding isolated colonies and gamma (γ) hemolytic bacteria produce no hemolysis around colonies.

Noting the Observable Characteristics of Bacteria

Here are the more common culture characteristics of bacteria on agar plates:

1. **Odor Production**: Some bacteria produce a unique aroma (gas) as a result of their metabolic processes. An astute microbiologist will always make note of the specific aroma of the organism under study. Examples of aroma are ammonia (NH3), rotten egg (H2S), alcoholic (-OH), fecal, etc.

2. **Pigment Production**: Pigments produced by bacteria are either cell bound (limited to cells and the colony), extracellular (pigment is released into the medium) or both. The color of the pigment should be noted, as this characteristic could be very helpful in the identification of the organism.

3. **Colonial Morphology**: This category includes the colony density, texture, elevation, cohesiveness, shape and margin. Note: Although we do not require you to learn these terms by heart, it is a good idea to get familiar with them.

a. **Colony density**: Hold the plate in front of a light source to determine if the colonies are clear, opaque (no light passes through the colony) or translucent.

b. **Colony texture**: This is best observed when direct light is reflected off the colonies. The texture is usually either smooth (even surface) or rough (irregular, nonsmooth surface).

c. **Colony elevation**: Colony elevation can be observed under direct light. It can be either flat, raised, convex, pulvinate, or umbonate .

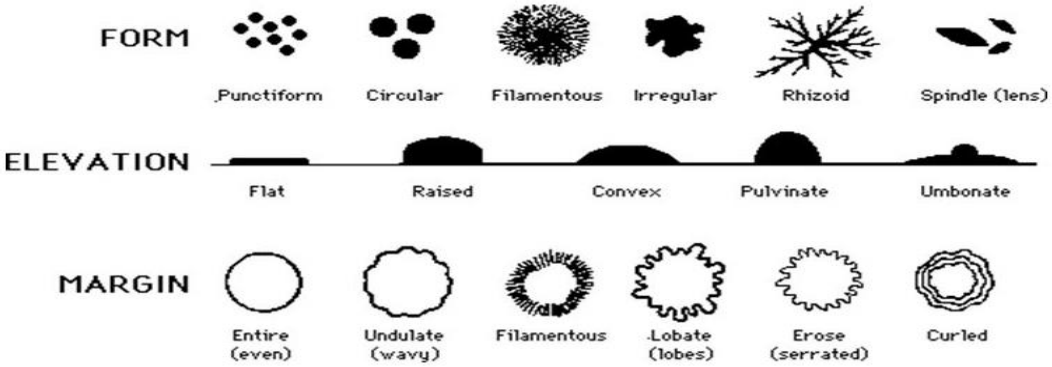
d. **Colony cohesiveness**: This can be grouped into stringy, creamy, mucoid and dry. The dry colonies can be lifted off the plate entirely and they are hard to suspend in liquid.

e. **Colony shape**: If viewed from above, the colony shape could be categorized as punctiform, which are very small pointed shapes, filamentous, spindle-shaped, irregular, circular, or rhizoid.

f. **Colony margin**: Colony edge or margins could likewise be described as entire, undulate, filamentous, curled, lobate or erose.

4. **Colony size**: The relative diameter (in mm) of average individual colonies growing on a certain medium can be a useful characteristic, especially when one is comparing different species.

Colony Morphology



Exercise 13

Observing Agar Plates:

1. Examine the provided plates and count the number of colonies on each. In the chart provided, record the number of colonies observed.

2. In the chart, describe the appearance of these colonies according to the descriptive terms for colonial morphology that were given at the end of the handout.

3. Draw sketches of colonies from each plate to illustrate colonial types.

	Number and Appearance of Colonies on Nutrient Agar			
plate	Type of Specimen	Number of colonies on plate	Word description of colonies	Sketch of some colonies
Plate 1				
Plate 2				
Plate 3				

Module 14

DETERMINATION OF VIABLE COUNT OF MICROORGANISMS

As part of daily routine, the laboratory microbiologist often has to determine the number of bacteria in a given sample as well as having to compare the amount of bacterial growth under various conditions. Enumeration of microorganisms is especially important in dairy microbiology, food microbiology, and water microbiology.

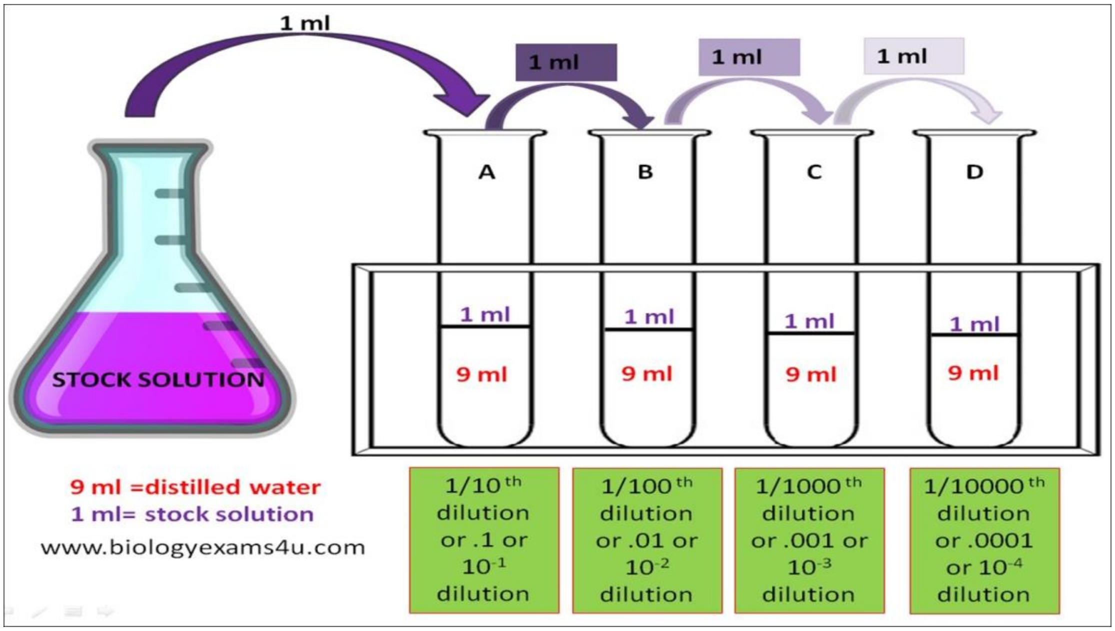
The plate count (viable count)

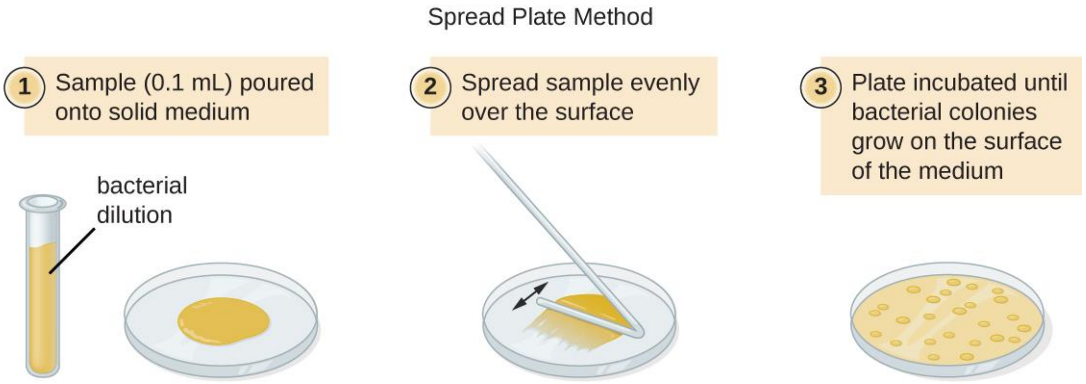
The number of bacteria in a given sample is usually too great to be counted directly. However, if the sample is serially diluted and then plated out on an agar surface in such a manner that single isolated bacteria form visible isolated colonies, the number of colonies can be used as a measure of the number of viable (living) cells in that known dilution. However, keep in mind that if the organism normally forms multiple cell arrangements, such as chains, the colony-forming unit may consist of a chain of bacteria rather than a single bacterium. In addition, some of the bacteria may be clumped together. Therefore, when doing the plate count technique, we generally say we are determining the number of Colony-Forming Units (CFUs) in that known dilution. By extrapolation, this number can in turn be used to calculate the number of CFUs in the original sample.

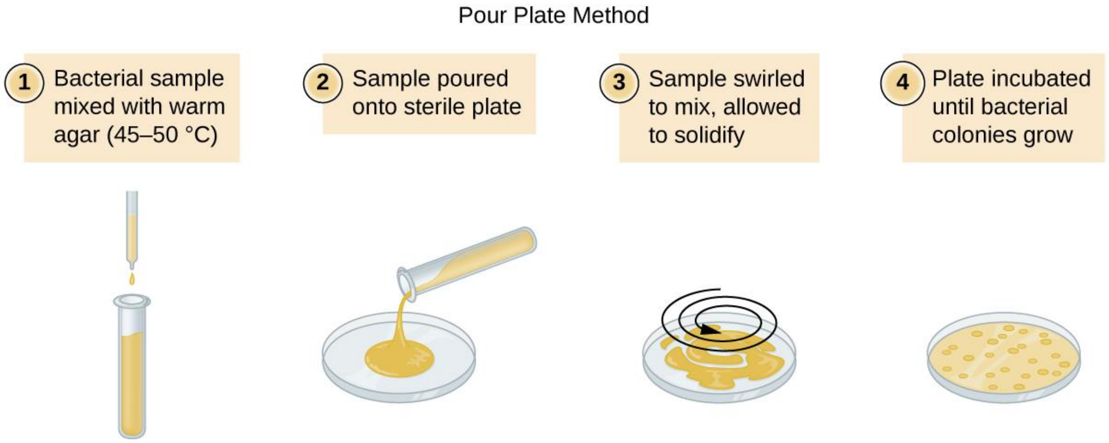
Normally, the bacterial sample is diluted by factors of 10 and plated on agar. After incubation, the number of colonies on a dilution plate showing between 30 and 300 colonies is determined. A plate having 30-300 colonies is chosen because this range is considered statistically significant. If there are less than 30 colonies on the plate, small errors in dilution technique or the presence of a few contaminants will have a drastic effect on the final count. Likewise, if there are more than 300 colonies on the plate, there will be poor isolation and colonies will have grown together.

Generally, one wants to determine the number of CFUs per milliliter (ml) of sample. To find this, the number of colonies (on a plate having 30-300 colonies) is multiplied by the number of times the original ml of bacteria was diluted (the dilution factor of the plate counted). For example, if a plate containing a 1/1,000,000 dilution of the original ml of sample shows 150 colonies, then 150 represents 1/1,000,000 the number of CFUs present in the original ml. Therefore the number of CFUs per ml in the original sample is found by multiplying 150 x 1,000,000 as shown in the formula below:

The number of CFUs per ml of sample = The number of colonies (30-300 plate) X The dilution factor of the plate counted


1 ml
1 ml
1 ml
1 ml
A
B
C
D
1 ml
9 ml
1 ml
9 ml
1 ml
9 ml
1 ml
9 ml
STOCK SOLUTION
9 ml =distilled water
1 ml= stock solution
www.biologyexams4u.com
1/10th dilution or .1 or 10-1 dilution
1/100th dilution or .01 or 10-2 dilution
1/1000th dilution or .001 or 10-3 dilution
1/10000th dilution or .0001 or 10-4 dilution


Spread Plate Method
1 Sample (0.1 mL) poured onto solid medium
2 Spread sample evenly over the surface
3 Plate incubated until bacterial colonies grow on the surface of the medium
bacterial dilution


Pour Plate Method
1 Bacterial sample mixed with warm agar (45–50 °C)
2 Sample poured onto sterile plate
3 Sample swirled to mix, allowed to solidify
4 Plate incubated until bacterial colonies grow

Exercise 14

Choose the best correct answer of the following:

1. In pour-plate method, the medium should be maintained at what temperature?
   a) 37 degree C
   b) 67 degree C
   c) 45 degree C
   d) 0 degree C

2. Which of the following method can be used to determine the number of bacteria quantitatively?
   a) Streak-plate
   b) Spread-plate
   c) Pour plate
   d) Pour-plate and spread plate

3. Dilution of the mixed culture is a common step in all the three methods: the streak-plate,the spread-plate and the pour-plate technique.
   a) True
   b) False
   c)May be
   d)Can't say

4. the type of dilution used in viable count methods is
   a) 2-fold serial dilution
   b) 10-fold serial dilution
   c) 5-fold serial dilution
   d) 3- fold serial dilution

5. in streak plate method, we apply about
   a) 0.1 ml culture to the plate
   b) 1ml culture to the plate
   c) 10 µl culture to the plate
   d) 50 µl culture to the plate

Module 15

ASSAY OF AN ANTIBIOTIC AGAINST STANDARD ANTIBIOTICS

The microbiological assay of an antibiotic is based upon a comparison of the inhibition of growth of micro-organisms by measured concentrations of the antibiotics under examination with that produced by known concentrations of a standard preparation of the antibiotic having a known activity.

INTRODUCTION:

Two general methods are usually employed, the cylinder-plate (or cup-plate) method and the turbidimetric (or tube assay) method.

The cylinder-plate method (Method A) depends upon diffusion of the antibiotic from a vertical cylinder through a solidified agar layer in a Petri dish or plate to an extent such that growth of the added micro-organism is prevented entirely in a zone around the cylinder containing a solution of the antibiotic. The turbidimetric method (Method B) depends upon the inhibition of growth of a microbial culture in a uniform solution of the antibiotic in a fluid medium that is favourable to its rapid growth in the absence of the antibiotic.

The assay is designed in such a way that the mathematical model on which the potency equation is based can be proved to be valid. If a parallel-line model is chosen, the two log doseresponse lines of the preparation under examination and the standard preparation should be parallel; they should be rectilinear over the range of doses used in the calculation. These conditions should be verified by validity tests for a given probability. Other mathematical models, such as the slope ratio method, may be used provided that proof of validity is demonstrated.

Preparation of the standard solution

To prepare a stock solution, dissolve a quantity of the Standard Preparation of a given antibiotic, accurately weighed and previously dried, in the solvent specified, and then dilute to the required concentration as indicated. Store in a refrigerator and use within the period indicated. On the day of assay, prepare from the stock solution five or more test dilutions

Preparation of the sample solution

From the information available for the substance under examination (the "unknown"), assign to it an assumed potency per unit weight or volume, and on this assumption prepare on the day of the assay a stock solution and test dilution as specified for each antibiotic but with the same final diluent as used for the Standard Preparation. The assay with 5 levels of the Standard requires only one level of the unknown at a concentration assumed equal to the median level of the standard.

Test organisms

The test organism for each antibiotic is listed in Table 1, together with its identification number in the American Type Culture Collection (ATCC). Maintain a culture on slants of the medium and under the incubation conditions specified in Table 5, and transfer weekly to fresh slants.

Test Organisms for Microbiological Assay of Antibiotics

Antibiotic	Test Organism	ATCC1 No.
Amikacin	*Staphylococcus aureus*	29737
Amphotericin B	*Saccharomyces cerevisiae*	9763
Bacitracin	*Micrococcus luteus*	10240
Bleomycin	*Mycobacterium smegmatis*	607
Carbenicillin	*Pseudomonas aeruginosa*	25619
Chlortetracycline	*Bacillus pumilus*	14884
Erythromycin	*Micrococcus luteus*	9341
Framycetin	*Bacillus pumilus*	14884
	Bacillus subtilis	6633
Gentamicin	*Staphylococcus epidermidis*	12228
Kanamycin sulphate	*Bacillus pumilus*	14884
	Staphylococcus aureus	29737
Neomycin	*Staphylococcus epidermidis*	12228
Novobiocin	*Staphylococcus epidermidis*	12228
Oxytetracycline	*Bacillus cereus var, mycoides*	11778
	Staphylococcus aureus	29737
Polymyxin B	*Bordetella bronchiseptica*	4617
Spiramycin	*Bacillus pumilus*	6633
Streptomycin	*Bacillus subtilis*	6633
	Klebsiella pnumoniae	10031
Tetracycline	*Bacillus cereus*	11778

Exercise 15

You are provided with standard antibiotic (1000 µg/ml) and test antibiotic of the same class but its concentration is unknown, calculate theconcentration of the test antibiotic?

1- Measure the diameter of each inhibition zone (given the symbol X) for each concentration of both test and standard antibiotics.

	Conc(µg/ml)	*Log C*	*a(mm)*	*b(mm)*	*x(mm)*	x^2
S1						
S2						
S3						
T						

2- Draw a curve between Log C and x^2 *and calculate the concentration of the given antibiotic*

Attendance sheet

	Subject	Date
1-	**Safety management.**	
2-	**Identification of bacteria under microscopy and Smear Preparation.**	
3-	**Simple stain.**	
4-	**Gram-positive bacteria stain (Staphylococcus, Sarcina and Bacillus).**	
5-	**Gram-negative bacteria stain (Pseudomonas, E. coli, Neisseria).**	
6-	**Mixture of Gram-positive and Gram-negative bacteria stain .**	
7-	**Identification of candida albicans.**	
8-	**Ziehl-Neelsen Stain For Acid-Fast microorganism (T. B.)**	
9-	**Staining of spores (Bacillus).**	
10-	**Wide distribution of microorganisms, Distribution of microorganisms in environment.**	
11-	**Different culture media.**	
12-	**Streaking techniques.**	
13-	**Isolation of pure colonies on nutrient agar and on blood agar.**	
14-	**Determination of Viable Count of microorganisms.**	
15-	**Assay of an antibiotic against standard antibiotics.**	